The Assessment of Contemporary and Recent Biological Markers of Human Exposure to Fluoride

BY

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Declaration

I hereby declare that the work presented in this thesis is entirely my own and that, to the best of my knowledge, has never been published or presented for the award of any other degree or diploma of the University of other institute of higher education.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
BAQ	Biomarker Acceptability Questionnaire
BOS	Bristol Online Survey
BMI	Body Mass Index
DUFE	Daily Urinary Fluoride Excretion
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
FUFE	Fractional Urinary Fluoride Excretion
FCT	Federal Capital Territory
FEQ	Fluoride Exposure Questionnaire
FFQ	Food Frequency Questionnaire
GC	Gas Chromatography
HFA	High Fluoride Area
HMDS	Hexamethyldisiloxane
IRB	Institutional Review Board
ISE	Ion Selective Electrode
IMD	Index of Multiple Deprivation
LFA	Low Fluoride Area
LGA	Local Government Area
NHS	National Health Service
NRC	National Research Council
NIOSH	National Institute of Occupational Safety and Health
PLASVIREC	Plateau State Virology Research Centre
PCR	Polymerase Chain Reaction
SPSS	Statistical Package for Social Sciences
SCHR	Steering Committee for Humanitarian Response
TISAB	Total Ionic Strength Adjustment Buffer
TDFI	Total Daily Fluoride Intake
TDDFI	Total Dietary Daily Fluoride Intake
WHO	World Health Organisation

ABSTRACT

Background: Excessive or deficient intake of biologically available fluoride (F) can be primarily monitored using fluoride biomarkers such as urine, plasma, saliva for short-term exposure and hair and nails for long-term exposure, however, these biomarkers have not been fully investigated.

Main aim: The overall aim of the study was to find the most acceptable, feasible and reliable biomarkers of exposure to fluoride among contemporary and recent biological markers. The overall aim was investigated by undertaking two separate studies.

Methods: In the UK, children aged 4-5 years and their parents were selected through primary schools in Middlesbrough and Teesside University as well as Newcastle University. Participants completed either a hard copy of questionnaire or participated in an online survey. A few of the participants consented to provide samples of the biological marker which was analysed for fluoride. In Nigeria, sixty healthy children (4-5y) and their parents (≥20y) in low- and high- F water areas (LFA, HFA, respectively) participated. Total daily dietary F intake (TDDFI) was assessed by food frequency questionnaire coupled with analysis of F content of food and drinks consumed by either a direct method (Martinez-Mier *et al*., 2011) or an indirect method (Whitford, 1996). Toothpaste ingestion was assessed through questionnaires. Total Daily F Intake (TDFI) was calculated from TDDFI and toothpaste ingestion. Biomarker samples including 24-h urine, plasma, saliva, hair, fingernail and toenail clippings were collected, prepared and analysed for F contents. Participants also completed Biomarker Acceptability Questionnaire (BAQ) questionnaires for themselves and their children. Data obtained were analysed using SPSS.

Results: For the qualitative study, in the UK, a combined 59%, 60%, 47%, 67%, 71%, 71% and 32% would find collection of samples of 24-h urine, spot urine (one day), spot urine (2 days), saliva, blood, nails and hair, respectively, perfectly acceptable or slightly acceptable among adults whereas for children a combined 37%, 52%, 42%, 50%, 38%, 73% and 50% was found. However, in Nigeria, participants were indifferent about the acceptability of the various biological markers to use in measuring exposure to fluoride among adults and children. However, based on behavioural interest to future use among adults and children, they were more likely to provide blood samples. For the quantitative study conducted in Nigeria, the mean (SD) TDFI among children was 0.075 (0.036) and 0.277 (0.184) mg/kgbw/d in low- and high- fluoride water areas, respectively, while the corresponding

values in adults were 0.036 (0.020) and 0.125 (0.093) mg/kgbw/d. The contribution to the TDFI was mostly from the diet in the low- and high- fluoride water areas. In children, there was a strong correlation between TDFI and DUFE as well as with plasma fluoride concentration, a moderate relationship between TDFI and fingernail as well as with toenail fluoride concentration, and a weak correlation between TDFI and saliva as well as with hair fluoride concentration. In adults, there was a strong correlation between TDFI and DUFE, plasma and hair fluoride concentration, a moderate correlation between TDFI and fingernail as well as with toenail fluoride concentration.

Conclusion: For the qualitative study, in Nigeria blood was perceived the most acceptable biomarker of exposure to fluoride among adults and children whereas in the UK, nail was perceived as the most acceptable biomarker of fluoride exposure for both age groups. However, adults in the UK would still be willing to provide blood samples due to previous experience in giving such sample. For the quantitative study, among contemporary and recent biomarkers, plasma is a more reliable biomarker since it reflects total daily fluoride intake in both children and adults even when exposure is very low. Although, the urine sample can be considered in place of plasma where there is difficulty in obtaining samples. Whole saliva is not a good biomarker of exposure to fluoride in adults and children. For long-term exposure, nail sample could be more reliable in both children and adults.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

It is well established that fluoride, at low levels, can control dental caries. Early investigations revealed that the fluoride intake corresponding to consumption of 1 mg F/l water fluoride was about 0.05-0.07 mg per kg bw per day (McClure, 1943); a range which was then suggested as optimal fluoride intake to prevent dental caries (Ophaug *et al*., 1985). However, a systemic fluoride intake of greater than 0.1 mg/kg bw/day during enamel formation might lead to the development of dental fluorosis (Burt and Eklund, 2005). Chronic exposure to a very high dose of fluoride (10-20 mg/day) might result in skeletal fluorosis (EFSA, 2013), which has been reported in different parts of the world including India, China, Kenya, Tanzania, Nigeria (Awadia et al., 1999; Cao et al., 1997; El-Nadeef and Honkala, 1998; Khan et al., 2005; Choubisa et al., 2009). However, a majority of the committee of the National Research Council in a recent report concluded that exposure to fluoride in drinking water containing 4 mg/l or more for a long period might increase the rate of bone fractures in the population (NRC, 2007). It has been reported that major contributions to fluoride intake include artificially or naturally fluoridated water, food and drinks prepared with such water, dietary supplements e.g. fluoridated milk; non-dietary fluoride supplements e.g. fluoridated toothpaste, mouth rinses and gels; and other caries prevention agents such as tablets (Zohoori and Rugg-Gunn, 2000).

Urine is the main route of removal of fluoride from the body (around 60% in adults, 45% in children) (Villa *et al*., 2010). Approximately 10% of total daily intake of fluoride is eliminated through faeces (Ekstrand *et al*., 1994) and the amount of fluoride excretion is directly influenced by the amount of fluoride intake (Maguire and Zohoori, 2013). Due to the differences in systematic intake of fluoride and body elimination, the fluoride balance in the body can be positive or negative; positive when intake is greater than elimination and negative when excretion from the body is greater than intake. In addition, factors affecting metabolism of fluoride, including specific drugs, high altitude, some respiratory diseases, metabolic disease and level of physical activity, will affect the balance of fluoride in the body. There has been extensive documentation on the balance between fluoride exposure and the decrease in the prevalence of dental caries as well as associated increase in dental fluorosis prevalence (Burt and Eklund, 2005). Estimation of total daily fluoride intake is crucial when suggestions of fluoride use are being considered for dental caries prevention

while reducing fluorosis risk. To quantify fluoride intake from the diet, several dietary assessment methods have been used including: market basket food collection (Ophaug *et al*., 1980), 7-day food record (Schamschula *et al*., 1988), 3-day food diary (Maguire *et al*., 2007), duplicate-diet collection (Franco *et al*., 2005), food frequency questionnaire (Miziara *et al*., 2009) as well as fluoride exposure questionnaires (Martinez-Mier and Soto-Rojas, 2010). Estimation of fluoride in toothpaste is also difficult but valid and reproducible methods have been used in measuring and analysing the amount of toothpaste dispensed during tooth brushing and expectorated saliva by some authors (Maguire *et al*., 2007; Franco *et al*., 2005). These methods are usually time consuming, expensive and require a high level of expertise. The increasing movement of processed foods and drinks across water fluoridation boundaries has resulted in a halo effect, making it more difficult to quantify total fluoride intake (Maguire and Zohoori, 2013). Therefore, the use of biomarkers, including blood, bones, teeth and urine could be helpful. In addition, fluoride concentration in saliva, breast milk and sweat might reflect concentration of fluoride in blood and nail fluoride concentration (finger and toe) and hair might reflect past blood fluoride concentration and the body burden of fluoride (Rugg-Gunn *et al*., 2011). Studies have been conducted both in animals and humans to provide evidence for the accuracy of these biomarkers in determining fluoride exposure (Buzalaf *et al*., 2004; Schamschula *et al*., 1985; Furlani *et al*., 2001; Czarnowski and Krechniak, 1990). Recently, Buzalaf and Whitford (2011) found a better correlation between fluoride intake and nail fluoride compared with urine fluoride. Urine, fingernails, head hair, saliva and plaque in the study of Schamschula *et al*. (1985) also showed consistently increased fluoride concentrations with, though not proportionate to, increasing water fluoride levels. Few other reports have compared some of these biomarkers and the finding from those studies are inconsistent (Elsair *et al*., 1982; Susheela *et al*., 2013; Kono, 1997; Czarnowski and Krechniak, 1990). However, most of the studies did not take into consideration fluoride intake from all sources. Consequently, there is a need for further investigations comparing both the recent and contemporary biomarkers of exposure to fluoride to obtain the most reliable biomarker. Also, for a biomarker of fluoride exposure to be applicable in a large population, it should be easily collectible without objections from the donors.

1.2 FLUORIDE AND THE ENVIRONMENT

Fluorine is a gaseous halogen that does not occur in the elemental state in nature due to its high reactivity (WHO, 1994). It is also the most electronegative of all elements and occurs

naturally as fluoride after reaction with metallic elements or with hydrogen. Fluorides can be found naturally in air, water and the lithosphere where it represents about 0.06-0.09 % of the earth crust (WHO, 1994). Fluoride can be found in a vast variety of minerals which include: fluorspar, cryolite, apatite, mica, hornblende and some pegmatite. It can as well be found in the soil depending on the fluoride compound solubility, soil acidity and water presence (EFSA, 2013), although it only occurs at a very low concentration. Most of the fluoride available to humans originating from the sea is at a level of about 0.8-1.4 mg/l, whilst the fluoride level of water obtained from lakes, rivers and streams is usually below 0.5 mg/l. Some artesian wells in countries like China, India, Tanzania, Ethiopia, as well as Nigeria have recorded high fluoride concentrations in the water. The United Republic of Tanzania recorded fluoride concentrations as high as 95 mg/l (WHO, 1994). Fluoride in air, in gaseous or particulate form, comes from soil containing fluoride, volcanic dust or volcanic gas. Anthropogenic activities have also contributed to fluoride in the air. Some factories (aluminium production plants and phosphate fertilizer plants) contribute fluoride as high as 1.4 mg/l. The fluoride content of food items depends on the soil fluoride concentration and water used for irrigation and for processing, so it varies from place to place. An extensive review of food-borne fluoride shows that the fluoride content of unprocessed food is usually low between 0.1-2.5 mg/kg, although some foods like cereals, bananas, potatoes and sweet potatoes have been reported to have high values of 4.2 mg/kg and over (WHO, 1994). In some countries, the high fluoride water used in processing has been shown to raise the fluoride content of processed food above that of the original untreated food. Furthermore, the fluoride concentrations of beverages (soft drinks, fruit juices, drinks and tea) reflect those in the water used in their preparation with fluoride level between 0.1-1.4 mg/l, except for tea which contains up to 7 mg/l (Pang *et al*., 1992). Dental products have fluoride concentrations between 230 mg/l in mouth rinses to 12300 mg/l in acidulated phosphate fluoride (APF) gels applied topically to the teeth by dental professionals (Franco *et al*., 2005). Most dentifrices contain fluoride at 1000-1500 mg/l either as sodium fluoride or sodium monofluorophosphate (Whitford, 1994).

1.3 FUNCTIONS OF FLUORIDE

Fluoride is mainly found in the body within calcified tissues such as bones and teeth. Fluoride is not essential for tooth development but it is beneficial to the tooth, especially the enamel, during and after development. The effect of fluoride can be classified into a systemic effect and a topical effect. Most of the fluoride incorporated into the developing enamel

occurs during what is now called the pre-eruptive (systemic effect) period of enamel formation (Beltran and Burt, 1988). At the time of tooth eruption and the post-eruptive period that will take approximately two years, enamel is not quite completely mineralized and will undergo what is now called the topical effect. During the period of maturation of the enamel, accumulation of fluoride continues to occur at the outer surfaces. This fluoride is derived from the saliva, as well as exposure to fluoride-containing products such as dentifrices, food and beverages.

1.3.1 Systemic effect

The uptake of fluoride from circulation into the enamel occurs only during the formation of the tooth and it is incorporated into the hydroxyapatite of developing tooth enamel and dentine (EFSA, 2013), forming fluorohydroxyapatite, a more resistant apatite to acids generated from ingested sugars by oral bacteria, thereby making teeth less likely to develop caries (Buzalaf *et al*., 2011).

The partial substitution of fluoride for the hydroxyl group of apatite also alters bone mineral structure making it more stable and compact with an increased density and hardness (Hellwig and Lennon, 2004). Bone mass can be increased by the intake of sodium fluoride but the newly formed bone may lack normal structure and strength and this is more apparent in trabecular bone (EFSA, 2013). Fluoride also acts on osteoblasts and osteoclasts, giving a mutagenic effect on osteoblastic precursors (Bonjour *et al*., 1993) and enhanced function of osteoclasts (Taylor *et al*., 1990).

1.3.2 Topical effect

It should be noted that enamel and dentine are not only fluoridated during tooth formation. Weathererell *et al*. (1977) showed 8% replacement of hydroxyl groups by fluoride in the surface of the enamel of teeth in individuals living in areas with fluoridated drinking water and 3% replacement for individuals in non-fluoridated areas. It can, therefore, be inferred that the hydroxyl groups of the hydroxyapatite in mature dental enamel can exchange with fluoride from the fluid surrounding the enamel prisms and the outer surface of the tooth to form fluorapatite (Buzalaf and Levy, 2011). Fluoride from diet will also exert an anti-caries effect on erupted teeth, since fluoride present at constant low concentration will adsorb onto the crystal surface and keep it from dissolution even if the pH falls due to production of acid by oral bacteria (Featherstone, 1999). Calcium fluoride is produced when the fluoride in the mouth is supersaturated with calcium from saliva and calcium released from enamel surface,

adding to the fluoride reservoir within the mouth (EFSA, 2013). This fluoride has been shown to protect the enamel by inhibition of demineralization (Arends and Christoffersen, 1990).

1.4 ADVERSE EFFECTS OF FLUORIDE

Chronic excessive exposure to fluoride from drinking water, or in combination with exposure from other sources may result into skeletal fluorosis, dental fluorosis and non-skeletal manifestations or any combination of these effects (RGNDWM, 1993).

1.4.1 Dental fluorosis

Various studies have shown a correlation between increasing water fluoride concentration and increasing prevalence of dental fluorosis (Wang *et al*., 2004; WHO, 2006; Mandinic *et al*., 2009; Viswanathan *et al*., 2009; Mandinic *et al*., 2010). Moreover, the severity of dental fluorosis varies individually at the same level of intake (El-Nadeef and Hokala, 1998; EFSA, 2013), which has been attributed to genetic factors (Carvalho *et al*., 2009) and environmental factors (Dibal *et al*., 2008). Excessive exposure to fluoride during critical periods of amelogenesis of both primary and secondary teeth can result into the development of dental fluorosis (EFSA, 2013), which leads to enamel with lower mineral content and increased porosity (Alvares *et al*., 2009). In countries with a hotter climate, because of increased consumption of drinking water, a lower concentration of fluoride in the water can result in dental fluorosis (Cao, 1992). For example, a study in China (Chen *et al*., 1988) showed that 46% of the population exposed to drinking water containing 1 mg/l of fluoride developed dental fluorosis. In a study conducted in the northern part of Nigeria, 51% of children aged 12-15 years exposed to 0.0-0.4 mg/l fluoride in drinking water developed fluorosis (El-Nadeef and Honkala, 1998). Chuobisa *et al*. (1997) also reported dental fluorosis among a population exposed to 1.4 mg/l fluoride in drinking water. The extent of exposure from food and other sources such as toothpaste, mouth rinses etc. is not clear from these studies as only drinking water was considered as the source of exposure to fluoride. “It is possible that dental fluorosis will develop at concentrations in drinking water below 1.5 mg/l in areas where fluoride intake via routes other than drinking water is elevated” (Cao, 1992). Based on this effect, a tolerable upper intake level of 0.1 mg/kg bw/day for children up to the age of 8 years (EFSA, 2005) or 1.5 mg/day and 2.5 mg/day for children aged 1-3 and 4-8 years respectively have been recommended.

1.4.2 Skeletal fluorosis

Elevated fluoride intake can have a more serious effect on skeletal tissues. Chronic high levels of fluoride increase the risk of bone fracture and of the development of skeletal fluorosis when drinking water contains 3-6 mg/l of fluoride (WHO, 1996) and crippling skeletal fluorosis usually develops only when drinking water contains over 10 mg/l fluoride (IPCS, 1984).). Crippling skeletal fluorosis usually develops after many years of exposure, when drinking water contains over 10 mg/l fluoride. Dibal *et al*. (2012) reported clear manifestations of bowing of legs among children living in Langtang town, Plateau state Nigeria from birth and exposed to fluoride above 10 mg/l in drinking water. WHO (1994) reported that water fluoride levels of 4-8 mg/l in temperate climates have not been linked with any clinical signs or symptoms of skeletal fluorosis but in tropical areas (in some developing countries). The lack of cases of skeletal fluorosis in Europe possibly might be due to the low drinking water intake of fluoride in this region.

A maximum contaminant level goal for protecting against crippling skeletal fluorosis of 4 mg/l for fluoride was established by the US Environmental Protection Agency (USEPA) in 1986 and confirmed in 1993.

1.4.2 Other possible adverse health effects

Studies have shown other possible health effects that might result from exceptionally high exposure to fluoride. However, most of these reports are not based on robustly designed studies. Exposure to excessive fluoride intake or a prolonged systemic exposure to fluoride can be associated with adverse health effects including: hypothyroidism (Susheela et al., 2005; Yang et al., 2008; Hosur et al., 2012; Singh et al., 2014), a disorder of the thyroid system in which the gland does not produce sufficient thyroid hormone; effects on the development of the brain and other tissues (NMHRC, 2017); certain types of osteosarcoma (Bassin et al., 2006), and decreased birth rates (DenBesten et al., 1992; Freni, 1994). Numerous reports in humans have associated fluoride with reduced IQ (Anger et al., 1986; Zao et al., 1996; Calderon et al., 2000; Rocha-Amador et al., 2009; Yazdi et al., 2011). Li et al. (2004) reported fluoride as a toxic material for nerve development, which could have a negative impact on the neurobehavioral development of neonates. Other studies have shown harmful effects on the central nervous system affecting cognitive and autonomic functioning (Guo et al., 2001), olfactory and some cognitive functions (Calvert et al., 1998).

1.5 METABOLISM OF FLUORIDE

When fluoride is taken into the body, it is not metabolised but it "crosses cell membranes as HF following a pH gradient from the more acidic to a more alkaline compartment" (Buzalaf and Whitford, 2011). Its absorption, distribution and excretion are dependent on pH.

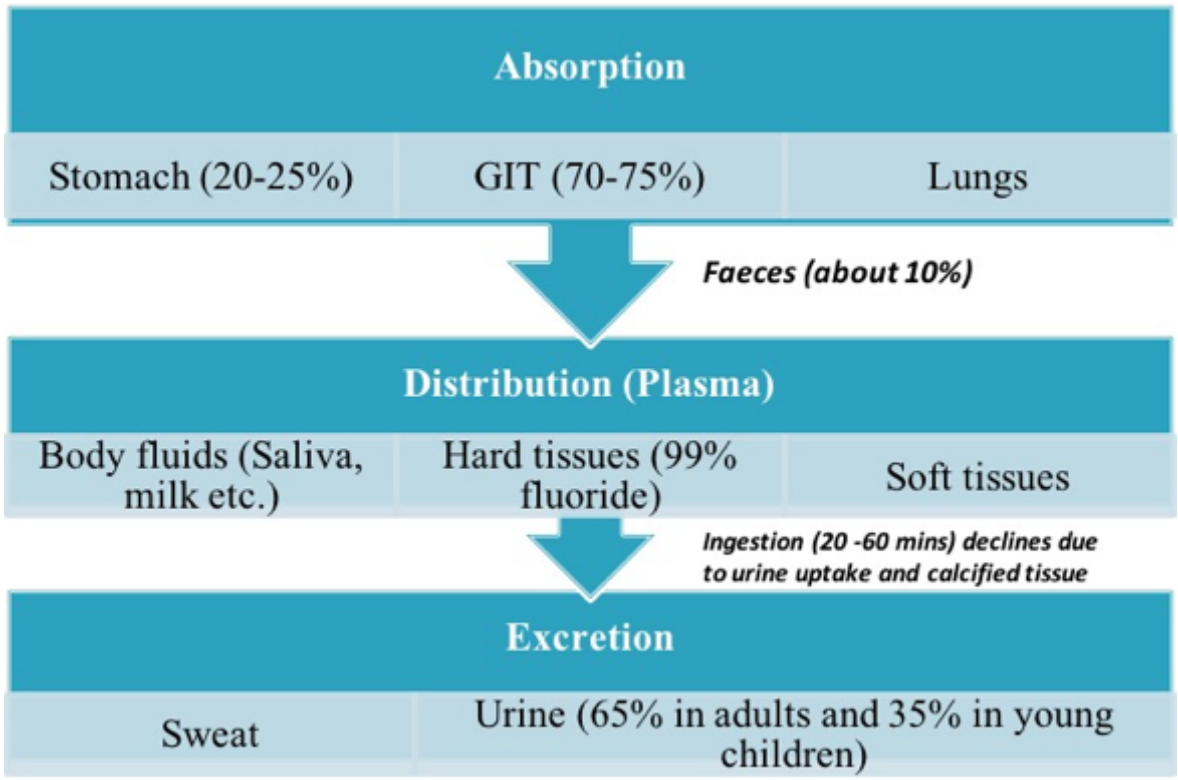

Figure 1.1 Fluoride metabolism

1.5.1 Fluoride absorption

Fluoride ingestion can be through dietary sources, e.g. water, food, beverages and fluoride supplements, and non-dietary sources, e.g. toothpastes, mouth rinses, gels and drops. In the absence of Ca, Al and Mg that form complex fluorides and sparingly soluble compounds, approximately 80-90% of ingested fluoride is absorbed from the gastrointestinal tract (EFSA, 2013). However, such absorption is reduced at high level of calcium and other cations that forms insoluble complex with fluoride. The absorption of fluoride across the oral mucosa is limited and probably accounts for less than 1% of the daily intake (WHO, 1994). Gastric absorption, distribution in the body and renal excretion are pH dependant. Therefore, the higher acidity of the stomach increases gastric absorption as undissociated HF and the remaining fluoride will be absorbed in the proximal small intestine as ionic fluoride (Venkateswarlu, 1994). Fluoride absorption occurs rapidly with a half-life of 30

min with stomach absorption accounting for 20-25% of ingested fluoride and 70-75% by the small intestine (Buzalaf and Whitford, 2011). The rate of absorption in the stomach is determined by gastric acidity (Whitford and Pashley, 1984), velocity of gastric emptying (Messer and Ophaug, 1993) as well as intake from foods and specific salts of fluoride ingested (Ekstrand and Ehrnebo, 1979; Trautner and Einwag, 1989). The fluoride not absorbed from the stomach will be absorbed by the proximal small intestine and the absorption is not affected by pH.

1.5.2 Fluoride distribution

1.5.2.1 Fluoride in plasma

The distribution of fluoride is through the plasma. "Plasma fluoride is the compartment from which fluoride is distributed to hard and soft tissues and for elimination from the body" (Buzalaf and Whitford, 2011). Plasma fluoride concentration peak is reached within 20-60 min after ingestion of a single dose and this is independent of the amount and nature of the fluoride ingested (Whitford *et al*., 2008). The concentration of fluoride in plasma returns to baseline within 3-6 hours because of uptake into the calcified tissues and urinary excretion (Whitford, 1994). The rapid excretion occurs through the renal system over a period of 4-6 hours. Fifty percent of the total fluoride absorbed is excreted in children below 3 years of age whereas about 90% is excreted in adults and children over 3 years (WHO, 1994). Fluoride balance in children can be positive or negative during the early months of life depending on whether intake is sufficient to maintain the plasma concentration that existed at the time of birth (WHO, 1994). The ionic form of fluoride, which is detectable by ion selective electrode and not bound to proteins and other components of plasma, is the form of interest in public health. In healthy adults, whose major source of fluoride exposure is diets, the numerical value of fasting plasma fluoride level is roughly equal to that of the drinking water if the fasting plasma fluoride concentration is expressed as µmol/l and the water is expressed as mg/l (Guy *et al*., 1976). Variations to this value can be attributed to individual differences in the rate of removal of fluoride by the kidneys and deposition in bones.

1.5.2.2 Distribution to tissues

In the soft tissues, fluoride absorbed is rapidly distributed through circulation to the intracellular and extracellular fluids where a steady state is established. Approximately 1% of the absorbed amount of fluoride is found in soft tissue. Whitford *et al*. (1979) reported

that the ratio of soft tissue fluoride to plasma fluoride in rats is between 0.4 and 0.9 with the exception in the pineal gland, kidney, brain, and adipose tissues. Diet, drugs, the level of physical activity or some metabolic diseases can alter the pH gradient thereby promoting the net influx of fluoride into and out of the cells (EFSA, 2013). Acidotic states can also result in increased plasma fluoride by a reduction of renal excretion (EFSA, 2013), although, in some exceptional cases, the kidneys can accumulate fluoride to higher concentrations compared to plasma.

1.5.2.3 Accumulation in the body

Approximately 99% of fluoride body burden is found in the skeleton where it is firmly but not irreversibly bound mainly as fluorohydroxyapatite (Whitford *et al*., 2008). The ionic fluoride concentration between soft tissues and hard tissues is directly related to the level of ionic fluoride intake (WHO, 2004). The total fluoride content of the human body amounts to 2-5 g and is dependent on age and fluoride exposure but the skeleton of a new born contains only about 5-50 mg of fluoride (EFSA, 2013). In bone, concentration of fluoride increases with age, past chronic intake, residence at high altitude and in the acidotic state. The increase is more rapid in women than in men, and higher in cancellous than in compact bones. In children, fluoride is taken up when forming bones and during the remodelling of bones, while in adults during bone resorption and remodelling. There is a greater percentage of fluoride retention in children than in adults (Ekstrand *et al*., 1994). The higher retention is because of the large surface area provided by the numerous and loosely organised developing bone crystallites, which increases the fluoride clearance rate from the plasma by the skeleton. Newman and Newman (1985) proposed that the fluoride uptake in bone is in stages. The first stage involves migration of fluoride into the hydration shells of bone crystallites where it is exchangeable due to the presence of extracellular fluid and can undergo net migration between the extracellular fluid and hydration shell (Whitford, 1989). The later stage is the incorporation into precursors of hydroxyfluorapatite and finally into the apatitic lattice itself (Whitford, 1994). Due to long term process of bone resorption, apatitic fluoride re-enters the circulating body fluid.

1.5.3 Fluoride elimination

1.5.3.1 Renal excretion

The kidneys are the main route of elimination of fluoride from the body. Renal clearance of fluoride is high compared to other halogens and directly related to urinary pH. Varying

values of the clearance of fluoride among individuals within a given study have been reported; 28 – 55 ml/min and 12 -71 ml/min by Waterhouse *et al*. (1980) and Schiffl and Binswanger (1982) respectively. The differences could be attributed to glomerular filtration rate, urinary pH and flow rate under certain circumstances. Some factors that lead to increased acidity of urine increase retention of urine, including: metabolic and respiratory disorders, composition of diet, altitude of residence and certain drugs (Whitford, 1994). Likewise, renal clearance is reduced, and hence tissue retention of urine increased, when glomerular filtration rate is depressed on a chronic basis. The amount of fluoride in plasma declines rapidly following ingestion due to excretion through urine and uptake by calcified tissues. Spak *et al*. (1985) suggested that children have lower renal clearance rates than adults because of fluoride utilisation by the children's developing bones. Various percentages of renal clearance of fluoride have been reported by different authors, for example, 65% in adults, 35% in children (Villa *et al*., 2010) and 30% in children by WHO (2004). The percentage of absorbed fluoride excreted by the kidneys can be as low as 10-20% in infants and young children due to the increased capacity of their bones to store fluoride (EFSA, 2013). Dietary and other factors change the acid-base balance of the body and a decreased urinary pH will reduce renal fluoride excretion and lead to higher body retention of fluoride (EFSA, 2013). Other factors such as impaired function and age-related factors that decrease the glomerular filtration will also decrease the excretion of fluoride (Torra *et al*., 1998; Jeandel *et al*., 1992).

1.5.3.2 Sweat

Early reports of the 1940s revealed that the concentration of fluoride excreted in sweat could nearly equal urinary fluoride excretion under hot humid conditions (Crosby and Shepard, 1957; WHO, 1994). More recent data obtained using ion selective electrodes showed that sweat fluoride concentrations are very low and close to plasma values. The study of Murray *et al*. (1991) showed that the F concentration in plasma and sweat was raised to 0.24 mg/l and 0.05 mg/l respectively after ingesting 10 mg of fluoride, but Whitford (1996) reported similar fluoride concentrations of plasma and sweat.

1.5.3.3 Faeces

It is estimated that approximately 10% of the daily fluoride intake that is not absorbed is excreted in the faeces (Buzalaf and Whitford, 2011). However, this percentage could rise to about 25% depending on some of the factors including 1) In the presence of Ca^{2+} diets,

unadsorbed calcium bonds with fluoride and migrates to the intestinal tract and 2) Where there is high concentration of fluoride in plasma, it can result in net migration of fluoride from systemic circulation into intestinal tract.

1.6 BIOMARKERS OF FLUORIDE

Measurements of fluoride in media such as air, water, food and beverages have been used to estimate the total body burden of fluoride in humans and for modelling human exposure by application of standardised assumptions based on individual behaviour, activity level, dietary choices etc. This is, however, becoming difficult due to continuous changes in the growth and behavioural pattern of humans, as well as changes in the environment which are influenced by its size and by the activities of the individuals living in it. Therefore, fluoride exposure may best be estimated using biological tissues and fluids when monitoring fluoride as a risk factor for dental and skeletal fluorosis.

Generally, biological markers have gained much attention for their use in environmental monitoring of individuals and populations exposed to chemical exposure. The use of biological markers in environmental health has been reported by various publications issued by the Board of Environmental Studies in Toxicology of the National Research Council of the USA (NRC 1987, 1989). The NRC defined biological markers as "*indicators signalling events in biological systems or samples*" and further described them as "*any biochemical, genetic, or immunological indicator that can be measured in a biological specimen*". The Technical Report on Fluorides and Oral Health (WHO, 1994) described the concept of biological markers of fluoride exposure. In this report, the WHO stated that a fluoride biomarker is of value primarily for identifying and monitoring deficient or excessive intakes of biologically available fluoride. Although the use of biological markers is not without its challenges, including interpretation of results appropriately, the ideal method used should be precise, sensitive, and specific.

Figure 1.2 reflects both environmental monitoring and biomonitoring: the upper part revealing environmental monitoring, the pollutant measurement and product breakdown in the different environmental media including air, water, food, soil and manmade objects, and the lower section revealing biomonitoring, the direct measurement of contaminants in the body.

Biomonitoring, has the advantage of being able to measure the body burden of fluoride from integrated exposure via all routes of exposure. The biological tissues and fluids reflect the

biologically available fluoride capable of causing dental and skeletal fluorosis and which is not susceptible to assumptions or models.

Biomarkers of fluoride can be classified into the following: biomarkers of susceptibility; biomarkers of effect and biomarkers of exposure (Mussali-Galante *et al*., 2013).

1.6.1 Biomarkers of susceptibility

These are usually an indicator of variations in an individual's dose-response to the same exposure pattern of a contaminant e.g. genetic factors, acid-base disturbances, renal disturbances, bone growth, and nutritional status. Many factors have been shown to affect the rate at which individuals respond to similar doses of fluoride and these factors influence individual fluoride metabolism. For example, two environmental factors are high altitude (Akpata *et al*. 2009) and temperature (Zohoori and Rugg-Gunn, 2000). Whitford (1997) also reported that high protein diets, high altitude and some respiratory disorders affected urinary pH. Other factors are genetic conditions (Carvalho *et al*., 2009), whilst malnutrition, metabolism of calcium and magnesium are also potential markers of fluoride susceptibility (Sutti, 1983).

1.6.2 Biomarkers of effect

These are a result of a previous exposure, a measure of the functional capacity of the system or an altered state that is recognised as impairment or diseases including dental and skeletal fluorosis. They are also a side effect of previous exposures. Dental fluorosis reveals the effect of ingestion of fluoride particularly in children up to age six (WHO, 1994). The level of fluorosis depends on the total fluoride dose, timing, duration of exposure and individual response. Dental fluorosis alone is not sufficient to reveal the degree of fluoride exposure in an individual; likewise, skeletal fluorosis. The severity may be dependent on individual features, dietary habits and calcium metabolism.

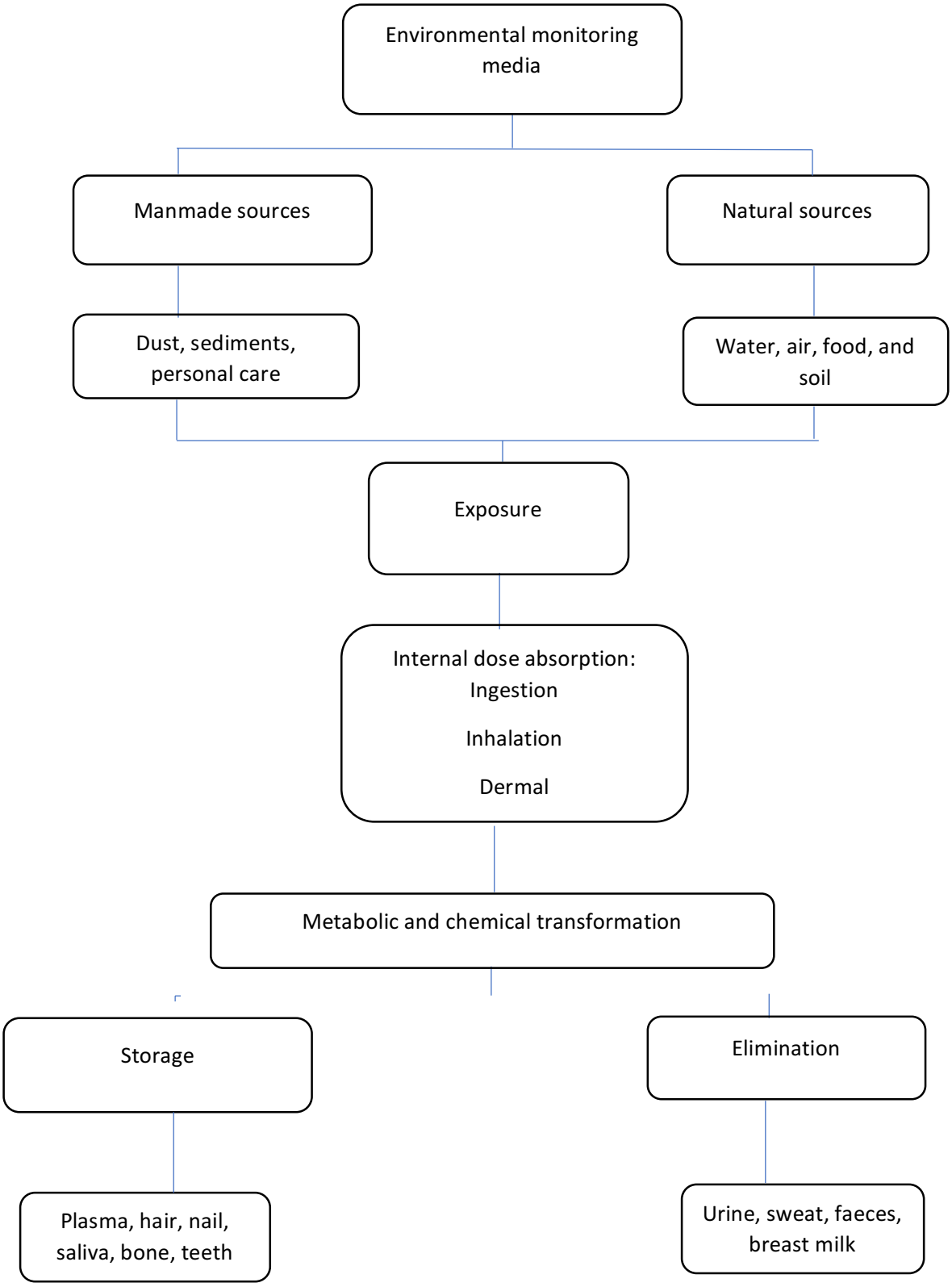

Figure 1.2: Environmental monitoring and human monitoring (modified from Needham, 2007)

1.6.3 Biomarkers of exposure

Biomarkers of exposure can be classified into historical, contemporary and recent markers (Rugg-Gunn *et al*., 2011). These are used to assess substances of external origin or metabolic derivatives of the product of an interaction between a biological agent and molecules. Biomarkers of exposure to fluoride contain a measurable amount of fluoride in biological tissues and fluids, which represent an individual's level of exposure. Biological samples such as teeth, bones, nails, hair, urine, blood or plasma and saliva have been useful in estimating exposure to fluoride (Vine 1994; Whitford *et al*. 1994; ATSDR 2003). However, only some biomarkers have been validated, although recently new analytical techniques have provided the opportunity to validate some biomarkers that have not been considered before. Only a validated biomarker can be used to assess the risk of exposure to contaminants in populations or in individuals. Some biomarkers indicate exposure that has occurred a few minutes or hours ago, while others reflect days, weeks or several months (Henderson, 1995). Plasma, saliva, serum and urine are short-term markers, while hair and nails reflect previous exposures of several weeks, months or years in the case of bones and teeth.

1.7 STATEMENT OF THE PROBLEM

There have been various studies relating the exposure of fluoride to dental caries prevention and to an increase in dental fluorosis prevalence (Kono *et al*., 1989; Khan *et al*, 2005; McGrady *et al*., 2012). Fluoride, however, has been revealed to be present in different kinds of foods, drinks and therapeutic products, and the determination of the amount of fluoride ingested is increasingly becoming difficult. Considering that only systemic fluoride is implicated in development of dental fluorosis, assessment of fluoride retention instead of fluoride intake seems to be more relevant (McDonnell *et al*., 2004). The balance of fluoride is a complex issue but it is of major importance to determine daily fluoride intake from all sources (Whitford, 1996). Conventional estimates are that about 75% of dietary fluoride comes from water and water-based beverages that contain more than 0.3 mg/L of fluoride (Freeze and Lehr, 2009) and 25% of total fluoride intake by man has a solid food origin. Therefore, water is by far the main source of fluoride intake for human beings (Formon and Ekstrand, 1996). However, other sources have been identified that have been shown to contribute significantly to the total intake of fluoride, such as fluoride-containing dental products, which are a non-dietary source of fluoride intake (Burt, 1992).

Considering that most foods contain low concentrations of fluoride, a study revealed in some African countries that certain types of food, for example, magadi, containing a salty, fluoride-rich condiment, have been claimed to have increased dental fluorosis in Tanzania and Kenya (Maybela *et al*., 1997). Such local factors have therefore made studies on metabolism of fluoride difficult as well as the widespread use of fluoride dental products and all other sources of exposure to fluoride. Consequently, it has become necessary to use biomarkers to study the absorbed dose that can lead to the development of dental fluorosis or skeletal fluorosis. In countries like Nigeria, India and China, the occurrence of skeletal and dental fluorosis is endemic geographical areas due to a high fluoride content in drinking water, which has led to a public health problem. There is therefore a need to monitor the effect of fluoride on these populations, so that governments and other health agencies may have data to take the necessary remedial action. However, this has been highly challenging due to the absence of an accurate and practical method for measuring combined fluoride intake both cross-sectionally and cumulatively (Mehta, 2013). This has resulted in the search for a reliable biomarker of exposure among the various biomarkers that have previously been studied. Among the biomarkers of fluoride exposure, special attention has been given to urine, hair and nails, since they are collected non-invasively, though hair has not gained much popularity.

The amount of fluoride in bodily fluids (e.g., urine, plasma, serum, saliva) are probably most suitable contemporary markers for assessing recent or current exposures to fluoride or fluoride balance (intake minus excretion). They are used for acute exposures due to rapid elimination of fluoride and measurements can be done shortly after samples are taken. The values obtained are not a direct measure of fluoride accumulation in the body, though they indicate the fluoride body burden (WHO, 1994). Urine has been used as an indirect indicator of fluoride intake for many years, and WHO has published provisional guidelines for estimating optimum or sub-optimum fluoride intakes (World Health Organisation, 2014). The 24-hour urinary fluoride output measurements which are independent of dietary habit, timing of meals and period of maximal fluoride intake (Marthaler, 1999) has been considered a useful contemporary biomarker of fluoride exposure in a population (Rugg-Gunn *et al.,* 2011). Fluoride concentration in plasma may give the best practicable indication of recent fluoride intake; ductal saliva fluoride concentration reflects those in plasma (Whitford, 1996) however, there is insufficient data on fluoride concentration of plasma across different age group to know the usual concentrations. While studies assessing the

time course of fluoride level in bodily fluids following fluoride compound ingestion typically involve timed collections of blood plasma or parotid ductal saliva, the amount of inorganic plasma fluoride varies based on the concentration of intake and several physiological factors, especially kidney function (Whitford, 1996). Studies in humans are difficult to conduct due to the burden and expense of blood collection (Buzalaf *et al*, 2002) but it has been used to provide pharmacokinetic data of fluoride (Ekstrand *et al*., 1994). Fluoride levels in ductal and glandular saliva closely follow the fluoride concentration in plasma, but at a lower level (about two-thirds of plasma concentration) (Whitford *et al*., 1999). However, their collection is difficult. Whole saliva has been found very useful provided that the factors that influence its utilization are considered (Boros *et al*., 2001).

The most studied recent fluoride biomarker is nail; less is known on the use of hair. Both biomarkers can be obtained non-invasively, although nail collection is more accepted by subjects (Sankhala *et al*., 2014). External contamination may be a challenge for the utilization of both biomarkers therefore, still needs to be better evaluated (Pessan and Buzalaf, 2011). Nails have been used to investigate acute (Buzalaf *et al*., 2004), chronic (Schamschula *et al*., 1985; Whitford *et al*., 1999), and sub-chronic (Correa Rodrigues *et al*., 2004; Buzalaf *et al*., 2006) fluoride exposure. Fukushima *et al*. (2009) investigated factors that might affect the relationship between exposure to fluoride and nail fluoride concentration. No guidelines have yet been established for the use of nails as an indicator of fluoride intake but it has shown high sensitivity and moderate specificity as a predictor of dental fluorosis (Buzalaf *et al*., 2012). Hair has been shown to be a useful indicator for chronic exposure to fluoride (Stolarska *et al*., 2000; Kokot and Drzewiecki, 2000) in endemic and low fluoridated areas (Parimi *et al*., 2013). It has also been shown to indicate children at risk of fluorosis regardless of the phase of tooth eruption (Mandinic *et al*., 2010). However, as with nails, no guidelines have yet been established for the use of hair as an indicator of fluoride intake.

No reports have compared fluoride amounts in urine, whole saliva, plasma, hair and nails (fingernail and toenail) but there have been some reports comparing fluoride in: urine and nails (Buzalaf *et al*., 2011), plasma and saliva (Oliveby *et al*., 1989), urine and saliva (Boros *et al*., 2001), hair and nails (Czarnowski and Krechniak, 1990). Although Schamchula *et al*. (1985) compared urine, fingernails, hair, saliva, plaque and enamel, plasma was not included in their study.

To summarise, there is no robust evidence on the usefulness of the various fluoride biomarkers yet. There is therefore a need to find the best marker among these contemporary and recent biomarkers of fluoride exposure based on several factors, including: practicality of collection and acceptability, practicality of analysis and validity of the biomarker.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Fluoride is effective in the prevention of dental caries but excessive ingestion of fluoride leads to the development of dental fluorosis. Several assessment methods have been used to measure total intake of fluoride (from diet and toothpaste ingestion) but this is ineffective in assessing the risk of dental fluorosis considering that only retained fluoride is associated with its development. Excessive or deficient intake of biologically available fluoride can be primarily monitored using fluoride biomarkers such as urine, plasma and saliva for short-term exposure and hair and nails for long-term exposures. Currently, there is insufficient evidence to conclude that tissues including nails and hair can be indicative of the amount of fluoride retained in the body due to problems of external contamination, methodology uncertainties and a lack of consensus about their sensitivity. There is a need for further studies to show the relationship between the amount of fluoride in these biological tissues and fluids and the intake dose and the associated risk of dental fluorosis in high and low fluoride water areas. Also, an ideal marker should be easily retrievable in a non-invasive way and easily collectible without objection from the donor.

This chapter is a critical narrative review of the available literature to contextualize the current study and identify the study gaps the study seeks to fill. The chapter explores the literature in two main areas including human exposure to fluoride and assessment of exposure through biomarkers. This was followed by the gap in knowledge and conclusion.

2.2 HUMAN EXPOSURE TO FLUORIDE

2.2.1 Sources of fluoride exposure

2.2.1.1 Air

Air is not a significant source of fluoride exposure, except in heavily industrialised areas. Fluoride concentrations of 0.5-3.8 $\mu g/m^3$ were reported in air samples collected in industrial cities in Germany in 1966 and 1965 (WHO, 1984). Some countries like the Netherlands and China have recorded exceptionally high values of 30 – 40 ng/m^3 and 16 – 46 ng/m^3 respectively (Slooff *et al*., 1988; Cao and Li, 1992).

2.2.1.2 Water

Fluoride is present in virtually all water in trace amounts, although higher concentrations have been associated with groundwater. In areas rich in fluoride-containing minerals, well water can contain up to 10 mg/l of fluoride with 2800 mg/l as the highest natural value reported in Lake Nakuru, Kenya (WHO, 2006). Seawater contains 0.8 – 1.4 mg/l and fluorides obtained in lakes are usually below 0.5 mg/l (WHO, 1994). Waters in European countries have generally low fluoride concentration but there are large regional differences because of varied geological conditions. SCHER (2010) reported fluoride concentrations of 0.01-5.8 mg/l in the natural drinking water of European Union member states while for bottled drinking water, which is increasingly used as a substitute for tap water, the concentration of fluoride has risen-up to 8 mg/l (EFSA, 2005). Kramer *et al*. (1974) also reported fluoride content in some US states to be between 0.08 - 0.44 mg/l with the highest value in Ohio (1.27 mg/l). Whitford (1994) reported that 55% of the US population is given water with fluoride below the range recommended by the World Health Organization (WHO) (Donohue et al. 2010). Developing countries have a varied concentration of fluoride in the waters, which is high in some parts of Tanzania, Kenya, Ethiopia, and Nigeria. Other parts of the world affected include China, India and northern Thailand (WHO, 1994). The recommended concentration range is 0.7–1.2 mg/l depending on the average regional temperature.

2.2.1.3 Beverages

Fluoride concentration in beverages reflect those in the water used for preparation, and they range from 0.1 – 1.4 mg/l except tea that contains up to 7 mg/l. The exceptionally high fluoride values found in tea have been attributed to naturally high fluoride levels in tea plants or additives use during growth or fermentation (WHO, 1996). Tea contains very high amounts of fluoride: between 170 – 400 mg/kg dry weight of black and green tea made from young leaves. These values can be 2 – 4 times higher for brick tea (Schmidt and Funke, 1984). Also, older tea plants have a higher concentration than the younger ones (George, 1963). The concentration of fluoride in milk can vary depending on the source; human breast milk, cow's milk, and soya milk contain 0.02 mg/l, 0.02 – 0.005 mg/l and 0.96 mg/l respectively (Koperal *et al*., 2000; Lal *et al*., 2014). Fluoride levels in carbonated soft drinks and fresh fruit juices range from 0.009 – 0.931 mg/l (Pang *et al*., 1992; Cutrufelli *et al*., 2004; Zohoori and Maguire, 2014) but values could vary with reconstituted fruit juice and

other drinks depending on the fluoride level in the water used in processing the drink. Pang *et al* (1992) reported values as high as 1.7 mg/l in carbonated drinks. Infant formula fluoride concentration depends on the water used for preparation and could range from 0.49 – 1.53 mg/l when made with non-fluoridated water (0.02 – 0.53 mg/l F) and 0.49 – 1.53 mg/l with water fluoridated at 1 mg F /l (Nohno *et al*., 2011; Zohoori *et al*., 2012), while ready-to-feed infant milk contains 0.030 mg/l fluoride (Maguire *et al*., 2012). Infant formula prepared with distilled water has a low fluoride content between 0.01 – 0.05 mg/l.

Fluoridated milk acts in the same way as fluoride in water, both systemically and topically, in the prevention of primary teeth dental caries (Bian *et al*., 2003; Pakhomov *et al*., 2005) and permanent teeth (Weitz and Villa, 2004; Petersson *et al*., 2011). For example, a double blind clinical trial conducted in Glasgow among school children aged 4.5-5.5 years old given 200 ml milk containing 1.5 mg F on each school day, showed a 36% reduction in DMFT compared to a group given non-fluoridated milk (Stephen *et al*., 1984).

2.2.1.4 Food

The fluoride concentration of food from plant origin is inconsistent due to: the location where the plant is grown, soil type, distance from air contaminating source, the prevailing wind direction and intensity, the period of the year (wet or dry), weather condition at the time of sampling, whether the edible part of the plant is leaf, root or fruit, whether the plant is fertilised, method of food procession and preparation for eating (Pang et al. 1992). Food generally has a low fluoride content between 0.1-0.5 mg/kg but there is a variation when it is prepared with fluoridated water. The concentrations of fluoride in vegetables and fruit (grown where there is no pollution), eggs, fish and fish sticks are 0.02 – 0.2 mg/kg fresh weight, 0.1 – 0.29 mg/kg, 0.18 mg/kg and 0.48 – 1.91 mg/kg respectively (EFSA, 2013). The highest fluoride concentration in vegetables grown in the field are found in curly kale (up to 40 mg/kg fresh weight) and endive (0.3 -2.8 mg/kg fresh weight), likewise fish (0.1 – 30 mg/kg) (Slooff *et al*., 1988). Grains have about 1 mg/kg fluoride and winter wheat in Sweden contains 0.38 – 1.35 mg/kg, although it could contain 0.92 – 3.69 mg/kg where the dust and soil are believed to contain high fluoride (George, 1963).

Fluoridated salt is often added to food either during or after cooking. Fluoridated salt contains 200 – 250 mg F/kg majorly in the potassium fluoride form. The value depends on national regulations and its use is sometimes restricted. Fluoridated salt increases ambient oral fluoride level right through life in a similar way to water fluoridation. Afssa (2003)

reported the amount of fluoridated salt ingested per person per day in France and Germany to be 3 g and 2 g respectively.

2.2.1.5 Dental products

Dental products that contains fluoride such as toothpastes, rinses, tablets and gels, but not considered a dietary fluoride source, can elevate the total fluoride intake especially when inappropriately used (Burt, 1992). They may contain relatively low F concentrations, e.g. 0.25-1 mg/tablet, 1000-1500 mg/kg toothpaste, or high fluoride levels, e.g. 10000 mg/l in liquids and 4000-6000 mg/kg in gels which are applied topically to the teeth by dental professionals.

2.2.2 Fluoride intake

Diet, unintentional toothpaste ingestion and fluoride supplements are the main sources of fluoride intake.

2.2.2.1 Dietary intake

The average daily dietary fluoride intake is approximately 0.5 mg or 0.04 – 0.07 mg/kg bw/day for young children with optimally fluoridated drinking water supply, as reported by earlier researchers (McClure, 1943; Ophaug *et al*., 1985). However, a recent study showed that breastfed infants have a low fluoride intake of approximately 0.3 – 1.6 µg/kg bw/day (Fomon *et al*., 2000). Whitford (1994) showed that human breast milk provides less than 0.01 mg/day. Wiatrowski *et al*. (1975) reported a dietary intake of 0.32 mg/day of fluoride for infants in the age group of 1 – 4 weeks, an increase in this value in the subsequent month due to increasing food intake, and a total of 1.23 mg/day for infants 4 – 6 months of age. This intake, when expressed per kg of body weight, ranged from 0.07 mg/kg in new-borns to 0.16 mg/kg in 6 months old babies (Wiatrowski *et al*., 1975). Intake of fluoride by infants is mainly dependent on whether they are breastfed with milk or formula (Ekstrand *et al*., 1984).

When an infant begins to eat, at about 6 months of age, fluoride intake is between 0.2 – 0.5 mg/day, depending on drinking water fluoride concentration or water used in food preparation, although there is a slight increase in the intake with two-year-olds (Ophaug *et al*., 1980; Debeka *et al*., 1982). These studies indicate an average of 2.8 mg/day for 2 years old children, but where water is fluoridated, the value is 0.9 – 6.7 mg/day (Whitford, 1994).

Estimation of daily fluoride intake conducted in Japan on infants 3-8 months old showed that the intake increased with age, being 0.166 mg, 0.202 mg and 0.266 mg for 3-4, 5-6, and 7-8 months, respectively, and the mean average intake by body weight was 0.023-0.029 mg/kg bw/day (Tomori *et al*., 2004). The intake increased as the babies grew older and this was associated with an increase in grains consumed which had a high fluoride content (0.30 ug/g) (Tomori *et al*., 2004). However, the value was lower than the optimum level of 0.05-0.07 mg/kg bw/day, making the infant at risk of dental caries. Zohoori and Rugg-Gunn (2000) reported a mean intake of 0.390 mg/day for 4 years old Iranian children residing in low fluoride areas but reported lower intakes of 0.107 ± 0.054 and 0.024 ± 0.015 in fluoridated and non-fluoridated areas of North-East of England respectively for infants whose main source of fluoride was their diet (Zohoori *et al*., 2014). In a study conducted among 2 – 6 years old children in Brazil, the average fluoride intake (Mean +/- SD) from solids, water and other beverages was 0.008 ± 0.005, 0.011 ± 0.004, 0.009 ± 0.014 mg/kg bw/day, respectively (Miziara *et al*., 2009). Kimura *et al* (2001) revealed that the mean dietary intake from food and drinks among 1 – 6 years old children living in a non-fluoridated area of Japan was 0.28 mg/day and 0.019 mg/kg bw/day, respectively, greater than that of children of western countries: 0.40-0.62 mg/day and 0.20-0.23 mg/day in fluoridated (0.7-1.0 mg/l) and non-fluoridated areas (<0.2-0.3 mg/l) respectively (Burt, 1992). This was attributed to the fact that participants consumed a lot of food with high fluoride e.g. fish, seaweed, and tea.

The average total dietary fluoride intake including tea and excluding drinking water for the adult population in the UK was recorded as 1.2 mg/day (EVM, 2001) but earlier studies reported 1.78 mg/day from both food and beverages and 0.4 mg/day from food only (Taves, 1983). In Sweden, Bruce (1981) also reported 0.4-1.0 mg/day and 2.1 – 4.4 mg/day for low fluoride and high fluoride areas respectively. The total fluoride intake/day depends on the amount of water consumed and degree of water fluoridation in a given area. For example, the mean fluoride content in the diet was 3 times greater between a high fluoride area (2.7 mg/d) and a non-fluoride area (0.9 mg/day) with 0.9 mg/l and 0.3 mg/l fluoride in the drinking water, respectively (Kramer *et al*., 1974). This study revealed no details on the composition of diets or the number of diets analysed, although, the fluoride content in the diet in different fluoridated areas varied and the differences did not correlate with the fluoride content of water. However, the authors attributed the differences in dietary fluoride

intake to processed food and canned food prepared in different locations with differing fluoride levels in the water used in preparing the food (Halo effect).

The study of Zohoori *et al* (2006) on dietary fluoride intake in 6-7 years old English children receiving optimally (< 0.7 mg F/l), sub-optimally (0.3-0.7 mg F/l) and non-fluoridated water (<0.3 mg F/l) showed that tap water, cordial prepared with the water, as well as certain cooked food like rice, pasta and vegetables contributed high amounts of fluoride in the optimal and sub-optimally fluoridated areas. In the optimal and sub-optimally fluoridated areas, there was also a large contribution from carbonated soft drinks and bread whereas the main contributors to dietary intake of fluoride in the non-fluoridated area were carbonated soft drinks and bread. Tap water may no longer be regarded as main source of fluoride intake because of increased pre-packaged drinks consumption particularly in developed countries where there is a shift to drinking beverage from drinking of water. The mean daily dietary fluoride expressed in mg/d (mg/kgbw/day) decreased for children receiving optimal, sub-optimal and non-fluoridated water, being 0.591 (0.027), 0.349 (0.016) and 0.188 (0.008) respectively. Similarly, a study conducted on 2-8 years old Japanese children in two areas with different fluoride levels in the water supply showed that the total daily fluoride intake was 0.025 mg/bw/day in a moderate fluoride area with 0.555 ppm F in the water and 0.0126-0.0144 mg/kgbw/day in a low fluoride area with 0.040-0.131 ppm F in the water (Nohno *et al*., 2000). This difference was also revealed in their typical Japanese diet like boiled rice and side dishes prepared with the supply water. In addition to the supply water which serves as an important source of fluoride, some studies have reported certain foods like tea, tobacco, pan masala (used with or without tobacco in India), and Magadi in Tanzania have been shown to contribute immensely to the total dietary fluoride intake (Yadav *et al*., 2007) and the evidence has shown that the leaching of fluoride from these foods increases several folds in acidic conditions of stomach (Yadav *et al*., 2007).

Studies conducted by Cressey *et al*. (2010) showed that animal products, e.g. meat, fish, chicken, eggs, and grain products are regular contributors to fluoride intake from diets across age groups and genders but the contribution of these food types tends to decrease with age. However, for children aged 5-14 years, contributions from bread reach peak and at 15-24 years, carbonated beverage contributions become significant with increasing contribution from tea consumption to fluoride intake as they increase in age. Also, alcoholic beverages are very important contributors for males in New Zealand than females while for non-alcoholic beverages a converse relationship (Cressey *et al*., 2010). The Halo effect, which

occurs when food and beverages are made with fluoridated water and then transported for consumption in areas that do not have fluoridated water, has also contributed to ingestion of fluoride from other sources, especially soft drinks and juice (Whitford, 1994). Most people in developed countries have taken these beverages as the liquid of choice to water and milk and the fluoride concentration of these liquids ranges from less than 0.1 to 6.7 mg/l. Whitford (1994) reported maximum intake values of fluids to be 1.42, 2.39 and 2.00 mg/day for 2 – 3 year olds, 7 – 10 year olds and 4 – 6 year olds, respectively. There are other factors that contribute to the estimation of total intake; among them is the water purifiers use and bottled water in the home, most of which are sold without removal of fluoride. Although some reports (Debeka *et al*., 1992; Chatha, 1986) showed fluoride level in bottled water to be below 0.3 mg/l, the impact will be shown when consumed in a fluoridated area. Studies have also revealed that there is higher than expected fluorosis prevalence in some sub-tropical and tropical countries (Warnakulasuriya *et al*. 1992), and WHO (1994) confirmed that the optimum fluoride level in the water of temperate regions may not be appropriate for other climate conditions as children could be consuming more water and be subjected to a daily dose greater than the recommended upper limit of 0.07 mg/kg bw/day. However, Lima and Cury (2003) found no statistically significant difference in fluoride intake during four seasons among 20-30 months old children in São Paulo, Brazil yet the quantity of diets consumed during the hot season was 19% greater than that during the cold season. Although they accounted that there was an un-sustained fluoride concentration in the water supplied and fluoride content of the water during the hot season was 50% lower, so the expected difference was annulled. Due to the multifactorial nature of sources of fluoride, it is not accurate to estimate total daily fluoride intake based on simply the drinking water fluoride level to relate fluoride intake and biologic effect or health outcome such as dental fluorosis or the quality of bone or it strength (Whitford, 1994).

2.2.2.2 Non-dietary intake

Fluoridated toothpaste: Fluoridated dentifrices have had an important impact on the reduction in children's caries rates (Franzman *et al*., 2004). However, dental products containing fluoride used for topical application of the teeth have become an important source of fluoride ingestion for children. The researcher is not aware of any evidence on ingestion of fluoride from toothpaste in adults. The results of several studies show that an average of 25% (10 – 100% individuals) of fluoride introduced into the mouth with toothpaste or mouth rinse is ingested in children (Bentley *et al*., 1999; Tan and Razak, 2005; De Almeida *et al*.,

2007). This percentage may be greater for young children that do not have good control of their swallowing reflex. “The average ingested fluoride amount with toothpaste by children whose water is fluoridated and have good control of swallowing and brush twice a day is approximately equal to the daily fluoride intake with the diet” (Whitford, 1987). Franzman *et al*. (2004) investigated tooth-brushing and dentifrice use among children aged 6 to 60 months from data obtained from the IOWA fluoride study and found that the use of dentifrice flavoured for children increased from 40% to 71% and the percentage of children using more than the recommended amount of dentifrice increased from 12% to 64% from 9 months to 60 months respectively. They associated the increase to the fact that children become less dependent on their mothers’ help with brushing as they grow up. A study conducted in 2-6 years old Brazilian children showed that 31.2% were at risk of developing dental fluorosis following high contributions of dentifrice to their total fluoride intake (Miziara *et al*., 2009). Dowel (1981) reported that 75% of children brushed at 18 months of age with on average 1 g of toothpaste (i.e. 1 mg fluoride for a 100 ppm F paste) per time, while Zohoori *et al*. (2012) reported the mean weight of toothpaste dispensed to be 0.67 ± 0.36 g for 4 – 6 years old children in England. The mean amount of fluoride ingested per tooth brushing session and per day was 0.017 and 0.0293 mg/kg body weight respectively. However, no significant difference was found between a low socioeconomic area and a high socioeconomic area despite the percentage of children who brushed twice in the high socioeconomic area being more. Another study was conducted by Zohoori *et al*. (2013) in children up to 4 years old living in fluoridated (0.64 mg/l) and non-fluoridated (0.04 mg/l) areas of Brazil, which showed no difference in the weight of toothpaste used by the children living in both areas when expressed by body weight (0.031 g/kg in fluoridated and 0.029 g/kg in non-fluoridated). Although the intake of fluoride from toothpaste ingestion was slightly greater in the non-fluoridated area (0.055 mg/kgbw/day) compared with the fluoridated area (0.037 mg/kgbw/day), this difference was not significant, with an overall total daily fluoride intake of 0.061 mg/kgbw/day in both areas. This report associated the differences in their intake to swallowing of toothpaste dispensed during tooth brushing, it could also be associated with the socioeconomic values as the children are the same age yet the difference. The fluoride ingestion from toothpaste is also affected by the fluoride content and the flavour of toothpaste, the toothpaste weight used, the tooth brushing frequency, the age and body weight of the child (Kobayashi *et al*., 2011). Forsman and Ericsson (1973) conducted an experiment on adsorption of fluoride from ingested toothpaste in rats and children, which also showed that polishing agents including calcium phosphate and calcium

carbonate prevented a considerable fraction of fluoride content from being absorbed in the small intestine whether present as F^- or FPO_3^{2-} as they bind with the fluoride in the small intestine.

Fluoride supplements: Dietary fluoride supplements provided in the form of drops, tablets or rinse supplements, intended for locations where concentration of fluoride drinking water is less than 0.7 mg/l, have been linked with increased dental fluorosis prevalence (Burt, 1992). This can be attributed to the prescription of wrong doses as well as usage in locations where water is adequately fluoridated. Szpunar and Burt (1992) reported that some small clinical trials in the US that provided evidence of caries preventive benefits have been criticised for study design faults and the way conclusions were drawn. As part of the IOWA fluoride study in the US, a study was conducted in children from birth to 96 months to assess the use of supplements. Fluoride supplement use was found to decline from 12 months (11%) to 96 months (4.7%), the use at 24-60 months was 40% lower than at age 12 months and continued to decline at 72-96 months (Hamasha *et al*., 2005). However, the estimated mean daily supplement ingested rose: 0.06 mg, 0.07 mg, and 0.18 mg for birth-12 months, 12-60 months and 60-96 months respectively.

2.2.2.3 Total daily fluoride intake (diet and toothpaste)

In the IOWA study (Levy *et al*., 2001), conducted between 1992 – 1995 among 1389 children on ingestion of fluoride from water, dentifrice and supplements by infants and young children from 0 – 36 months old, intake was highest from 0 – 3 months (0.075 mg/kg body weight) and reduced with increasing age. It was also obvious that the major contribution to the total daily intake was from water and fluoridated toothpaste use. However, it should be noted that, due to the variation in the fluoride content of water, formula fluoride intake may range from less than 0.4 mg/day to over 1.0 mg/day. This value is above the optimum range of 0.04 – 0.07 mg/kg bw/day, increasing the risk of fluorosis (Whitford, 1994). However, some earlier studies reported that dietary fluoride intake, when adjusted for body weight, tends to decrease with age relative to body weight as children develops from infancy towards their first and second birthday (McClure 1943; Adair and Wei, 1978; Burt 1992).

An earlier report revealed that the total fluoride intake/day depends on the amount of water consumed and the degree of water fluoridation in any given area. Kramer *et al*. (1974) showed varying fluoride intake in the diet by location including Oregon, California,

Madison and Milwaukee, 1 mg/d in the non-fluoridated area (0.08-0.44 mg F in water) while in the high fluoride area ranged between 1.73 to 3.44 mg/d (0.53-1.27 mg F in water). There was also a varied fluoride concentration in the diet from the different fluoridated areas and these differences did not correlate with the drinking water fluoride concentration. The authors related this to a halo effect as a great part of the diet consisted of processed, canned foods which may have been prepared in areas where the fluoride content was different from that in the locality. McClure (1943) estimated the intake of fluoride in children to be between 0.4 mg/d and 1.7 mg/d depending on their age, nature of diet and fluoride concentration of water, as stated earlier. Franco *et al*. (2005) showed no significant difference in total daily fluoride intake in children aged 22-35 months in four Brazilian cities based on their age and weights using a duplicate method of estimation. Although, socioeconomic status had influence in the fluoride intake: the total intake was high in children with low socioeconomic status (71.7% had a mean fluoride intake above the optimal dose of 0.07 mg/kgbw/day) compared to children with high socioeconomic status. The estimated daily fluoride intake associated with the use of 1500 ppm F toothpaste was 0.11 (0.09) mg/kgbw/day, which accounted for 70% of the total fluoride intake (Franco *et al*., 2005).

Considering the challenges associated with the duplicate method of sample collection for the estimation of fluoride intake, including parents' compliance and the cost of providing a double portion of the food consumed to be analysed particularly in poor countries where many live below a dollar per day, it is expedient to find an alternative method which would be more suitable for epidemiological surveys like the food frequency questionnaires (Levy *et al*., 2001, 2002, 2003). Miziara *et al*. (2009) evaluated total fluoride intake from the diet and dentifrices of 2-6 years old Brazilian children in a fluoridated area (0.6-0.8 ppm F) using a semi-quantitative food frequency questionnaire. They reported 0.064 (0.035) mg/kgbw/day as total daily fluoride intake, with a 56.3% contribution from dentifrice (0.036 (0.028) mg/kgbw/day) and the remainder from the diet (0.028 (0.007) mg/kgbw/day) including solids (11.8%), beverages (14.7%) and water (17.2%). This was in conformity with other studies (Pessan *et al*., 2005; de Almeida *et al*., 2007) particularly in that age group, as the use of dentifrices by children results in significant amounts of ingested fluoride particularly when application is not supervised by parents. In infants aged 0-12 months, where diet was the only fluoride source for 87% of the infants, the mean total daily fluoride intake was 0.107 (0.054) and 0.024 (0.015) mg/kgbw/day in fluoridated (0.97 mg/l) and non-fluoridated (0.19 mg/l) areas respectively (Zohoori *et al*., 2013).

2.3 DIFFICULTIES IN ASSESSMENT OF FLUORIDE EXPOSURE

Due to the halo effect (globalization of the food/drink industry; therefore, production of food/drinks in a fluoridated area and transportation to a non-F area and vice versa) as well as multiple sources of F intake (e.g. considerable intake from toothpaste ingestion in children), estimation of total fluoride intake which is the main risk predictor of dental caries is increasingly becoming difficult. Reasons are: limitation of dietary assessment methods (including reporting error, methodological error or bias associated with the techniques, over or under estimation of intake) as well as quantification of fluoride exposure from toothpaste ingestion.

2.3.1 METHODS OF ASSESSMENT OF DIETARY FLUORIDE INTAKE

2.3.1.1 Market basket

In this method, dietary intake of a certain nutrient is estimated from a collected representative diet of a study food group, which is based on shopping guidelines that originated from household consumption surveys showing the actual 14 – 28 days' consumption of different food items of the study group. Some early studies where fluoride exposure is virtually from naturally fluoridated water (McClure, 1943; Ophaug *et al*., 1980, 1985) have used market basket techniques to compute estimated intake. This method is usually very challenging to investigators due to the need for a survey of household food consumption for at least 14 days before analysis and inaccuracies when estimating intake by not taking into consideration household food waste and the differences in the type and amount of food consumed between adults and children.

2.3.1.2 24-hour dietary recall records

This method involves a structured interview, conducted between 20 to 60 min by a trained interviewer, whose purpose is to capture detailed information about all foods and beverages (including dietary supplements) consumed by the subject in a 24-hour period usually from midnight to midnight the previous day. The interviewer asks the subject the time of day, the food source, portion size of each food item and beverages which can be aided by food models and/or pictures. A more detailed information than first reported are being asked when appropriate in a way to provide a detailed and comprehensive report of all beverages and food eaten. It is quick, easy and depends on short-term memory, but may not be truly representative of the subject's usual intake as subjects tend to underestimate or under-report

serving sizes (MacDiarmid and Blundel, 1998; Pikholz *et al*., 2004). This method was used, for example, in New Zealand to estimate dietary fluoride intake by subpopulations to identify any population groups at risk of high fluoride intake (Cressey *et al*., 2010). This method is very limited and might be difficult to represent an adequate food intake of the participant. Similarly, it depends solely on the memory of the participant and might be lacking when they have memory issues. In this method, it might also be difficult estimating food quantities and food ingredients especially if participants eat in a food canteen.

2.3.1.3 Diet history

This is another method for assessing nutritional status where subjects are asked to remember their own food intake for a certain period (Martin-Monero and Gorgojo, 2007), usually the record of food consumed during 2 or 3 days. Walters *et al*. (1983) estimated the average dietary intake per person of fluoride in the United Kingdom by this method. The information, including details about the usual intake, types, amount, preparation method, frequency and timing as well as specific questions relevant for the survey carried out, is collected by a trained interviewer who needs to be highly qualified in nutrition and dietetics (Fagundez *et al*., 2015). It is important that the interviewer ensures the information provided are correct by cross-checking to verify data. The limitation of this method is its need for highly trained interviewers and the recall may not be precise. Considering that adults provide the information due to lack of cooperation of the children, the information provided by the parent therefore might not be representative of what the child has consumed thereby leading to over or underestimation. The method works if the participants can describe their typical daily intake which can be difficult when it relates to their children as there is the tendency where children would not want to report what they consume outside their home, mainly when it is unhealthy.

2.3.1.4 Food frequency questionnaire (FFQ) and semi-quantitative FFQs

An FFQ is usually self-administered (or interviewer-administered when the respondent literacy is low) and it is made up of a finite list of around 80 to 120 items of food and beverages with a response category to indicate the usual consumption frequency over the period; per day, per week or per month (Miziara *et al*., 2009), typically completed between 30 to 60 min. Portion size can be combined with information on food frequency by asking subjects to convert their usual consumption amount to some specific units, including plate sizes (1/2, 1/4, 3/4) and cup sizes, or they are shown portion size images to enhance reporting

accuracy. The FFQ is semi-quantitative when it includes reference portions previously determined for each food and is structured according to a food pyramid (Philippi *et al*., 1999, 2003; Colluci *et al*., 2004; Miziara *et al*., 2009). This method is used in many studies (Levy *et al*., 2001, 2002, 2003; Jackson *et al*., 2002; Broffitt *et al*., 2004; Hong *et al*., 2006) as an alternative to other more recognised methods of fluoride intake including the duplicate method, food diary, etc. (Omid *et al*., 2015). The FFQ has great benefits for providing information on queried food and beverages over a period. Information provided in a FFQ can be linked to databases (e.g. nutrient composition database) or database that translate food and beverages into food group equivalents. It also provides information about total dietary intake, capturing diets in retrospective case-control studies. The FFQ can be used to evaluate the effectiveness of interventions to diet change but its use as a sole evaluation method needs to be carefully considered due to misreport of diets between intervention group and control group. It has certain advantages including its low-cost, ease-of-use and efficiency to assess the usual diet of population groups (Slater *et al*., 2003; Miazira *et al*., 2009). It also allows the various components of diets to be separately compiled making it easier to identify dietary risk factors for aesthetically objectionable dental fluorosis, for example, when used by Miziara *et al*. (2009) to estimate fluoride ingestion among Brazilian children. However, an FFQ is a long questionnaire, which can lead to errors with estimating the serving size and needs constant updating with new commercial food products to keep pace with changing dietary habits (National Cancer Institute, 2014). Also, variations in fluoride concentration in specific batches of food items can be reflected in the fluoride intake thereby affecting data accuracy (Miziara *et al*., 2009). There might be an error when participants are completing the questionnaire. The questionnaire did not give the opportunity for reporting how the food is being prepared and ingredients used and therefore this might reflect on the fluoride intake. There is also a chance of underreporting or overreporting by participants, mainly when data are collected in the presence of friends or family as participant might want to show they eat healthily.

2.3.1.5 Observed food consumption

In clinical practice, this method is the most unused, but it is recommended for research purposes as a means of validating a dietary assessment method by providing an objective measure of dietary intake. The investigator observes the subjects directly and takes notes of eating behaviour during a defined period, usually short e.g. a one hour lunch break (Baglio *et al*., 2004), or longer e.g. 12 hours continuous monitoring (Branowski *et al*., 1986), when

items and amount of food consumed, received or given away, or spilled as well as the portion sizes are recorded. The meal consumed by the individual is weighed and the contents are calculated exactly. The method is characterized by having a high degree of accuracy on assessment of dietary intake and the social and physical context of dietary intake, and is useful for populations unable to record their own intake but it is expensive and needs time and effort. Shulman *et al*. (1995) estimated the desired daily fluoride dose by analysing data of food consumption from a stratified random sample of 7345 children studied during 1977-78 US Department of Agriculture Nationwide Food Consumption Survey.

2.3.1.6 Food diary (3 and 7 days)

With this method, a description of the food and the weight, including brand name, cooking and preparation methods, ingredients of home-made foods and the time of consumption, is recorded by the subject in a specially designed booklet which also has a space to record the weight of leftovers of all food and drink (Omid *et al*., 2015). The subject or the researcher weighs every item of food and drink before consumption or the weight of food and drink can be estimated using household measures, such as cups and spoons, food photographs/atlas (Nelson *et al*., 1997), and food models; which the researcher changes into weights that can be used to calculate food and nutrient intake. The length of collection period can be 3, 4, 5 or 7 days. However, a 7-day record has often been referred to as the gold standard compared to less detailed and demanding methods (Willett 1998). A 3-day diary has been used in investigating fluoride intake from beverages (Clovis and Hargreaves, 1988; Pang *et al*., 1992; Zohoori *et al*., 2006), conducted on two weekdays and one weekend day with an interview on the fourth day to ensure that all drink and food items consumed have been documented (Maguire *et al*., 2007; Zohoori *et al*., 2013). The food diary method estimates fluoride intake on the basis of food composition tables (Omid *et al*., 2015). This method is less burdensome and more practical for use with large population. However, Gersovitz *et al*. (1978) showed that subjects may forget what they have consumed when recording for more than 4 consecutive days, which may result in a decrease in reported intake, but this has been improved due to availability of a pocket-sized diary that is easily carried, allowing food and drinks to be recorded at the time of consumption thereby reducing the risk of omission (Omid *et al*., 2015). It has also been shown to be economical (Black, 1982), reasonably reliable (Hackett *et al*., 1984), approximates usual intake but is useful in determining daily variations (Rizek and Pao, 1990), and is a method which allows more detailed qualitative and quantitative information to be recorded including sources of fluoride

which might not be possible with another prospective method (duplicate method) (Zohoori *et al*., 2006; Maguire *et al*., 2007). Drawbacks such as misreporting or forgetfulness may be avoided if the subject records the food and drink prior to consumption (Omid *et al*., 2015) but may be affected by coding errors and/or non-inclusion of a food type in food composition tables (Basiotis *et al*., 1987). Other limitations include participant's limitation in reporting food quantities due to their level of education and there is the possibility that the weekly food log does not accurately represent the usual eating habit of the participants when they have the idea that the food they eat would be analysed and therefore be forced to eat healthier.

2.3.1.7 Duplicate method

This method involves keeping a duplicate portion of all food and drinks prepared, served and consumed during the period of study, normally over a 24-hour period (Dabeka *et al*., 1987; Nohno *et al*., 2006), 2 consecutive weekdays (Lima and Cury, 2003), 2 days including one weekday and one weekend day (Zohoori *et al*., 2013), 3 consecutive days (Brikhed, 1990; Guha-Chowdhury *et al*., 1990, 1996; Rojas-Sanchez *et al*., 1999; Kimura *et al*., 2001; Franco *et al*., 2005). Approximate amounts of ingested food are determined by household measures such as 'teaspoon' and 'cupful' (Zohoori *et al*., 2013). The researcher weighs the identical portion and records the weight before the food is chemically analysed. The weight record is very useful to check the completeness of the duplicate portion and serves as a way of checking the portion sizes. The researcher visits regularly every couple of days to monitor compliance and collect the food. This method is a gold standard, particularly for assessment of individuals, as it provides accurate nutrient intake data that is not subject to error inherent in data processing and does not depend on food composition from other sources such as food consumption tables (Basiotis *et al*., 1987; Nohno *et al*., 2006). Intakes are estimated based on actual food and drinks collected in the duplicate diets (Omid *et al*., 2015). However, it is expensive (Willett 1998; Gibson 2005), elaborate to execute, and imposes a high individual burden. Moreover, ethical dilemmas because of collection of food from people who may not have sufficient amount to eat, particularly from poor countries, make it unsuitable for epidemiological studies (Miziara *et al*., 2009). People might change their customary dietary habits to reduce the cost of duplicating meals (Omid *et al*., 2015). The estimate of intake can be influenced by bias in reporting if (i) any food and drink is omitted from the duplicate and (ii) the primary sources of food and drinks cannot be identified because all food and drinks are pooled (Omid *et al*., 2015). Zohoori *et al* (2013) found variations in estimated fluoride

intake within food and drinks collected during the week and those collected at the weekend among children up to four years living in Brazil, which was associated with more food consumed when children attended the nursery during the week. This might lead to inaccurate estimation due to overestimation when a 24-h weekend duplicate sample is used.

2.3.2 Method of assessment of fluoride intake from toothpaste ingestion

2.3.2.1 Questionnaire

This questionnaire contains questions about fluoride intake from toothpaste, such as: if the subject brushed their teeth, the frequency of tooth brushing, brand of toothpaste used, if the subject ingests the toothpaste on other occasions and the amount of toothpaste placed on the toothbrush (Miziara *et al*., 2009), which is usually presented diagrammatically. The amount of toothpaste on the brush has been presented, for example, as three different weights corresponding to 1.2 g, 0.6 g, or 0.3 g (Miziara *et al*., 2009) or as seven different weights (Broffit *et al*. 2004) corresponding to 1 mg, 0.875 mg, 0.75 mg, 0.5 mg, 0.25 mg, 0.125 mg and 0.063 mg. Alternatively, Levy *et al*. (1997) grouped toothpaste usage into four categories: very small amount (0.063 mg); recommended amount (0.125 mg and 0.25 mg); somewhat more than recommended (0.5 mg and 0.75 mg) and substantially more than recommended (0.875 mg and 1 mg). The subjects select the picture that best represent the amount of toothpaste loaded onto the toothbrush when cleaning the teeth. The estimate from the subject on the proportion of toothpaste ingested is then used to determine fluoride intake from toothpaste. The questionnaire may also contain questions on other sources of water consumed in the past, amount of water consumed by itself or mixed with other beverages and foods and the patterns of use of dietary supplements (Franzman *et al*., 2004). Where the subjects are children, the questionnaire contains some specific questions, such as who brushed the teeth and who placed the dentifrice on the toothbrush (Levy *et al*. 1997; Levy *et al*. 2000). This method can lead to over or under estimation particularly when parents are reporting on behalf of the child. However, this method has been used in an important longitudinal investigation between 1992 and 1995 from 8 Iowa hospitals among a cohort recruited at birth (Levy *et al*. 1997; Levy *et al*. 2000; Warren *et al*., 2001, 2002; Levy *et al*., 2001).

2.3.2.2 Toothpaste applied, toothpaste expectorated and mouth rinses

This is the gold standard method for estimating fluoride exposure from toothpaste (Franco *et al*., 2005; Zohoori *et al*., 2012; Zohoori *et al*., 2013). In this method, the researcher records the brand of toothpaste used and the labelled fluoride concentration, the amount of brushing, the frequency of brushing, and who places the toothpaste on the toothbrush. The participants, with the supervision of the researcher, are asked to dispense the toothpaste on the brush in the same way they do normally and brush their teeth according to their usual brushing habit with their normal toothbrush and toothpaste. The researcher weighs the toothbrush before and after dispensing the toothpaste onto the toothbrush and collects all expectorated saliva, rinse liquid and toothpaste mixture in a container. Any remaining toothpaste on the face and hands will also be collected and transferred into the container. All information is recorded on a toothpaste recording sheet. The quantity of water used for rinsing will be determined by measuring the water weight in the rinsing cup before and after use. The procedure is cumbersome and it requires a trained person who monitors the brushing routine and collects all samples for further analysis. It has the great advantage of ability to prevent error in estimation.

2.4 REVIEW OF BIOMARKERS OF EXPOSURE TO FLUORIDE

For a fluoride biomarker to be considered viable in a population, a relationship must be established between the biomarker fluoride concentration and the fluoride exposure or long-term intake. For the assessment of fluoride, exposure might be inferred, based upon water fluoridation in living area, instead of calculating actual intake (Rugg-Gunn *et al*., 2011). Several biological markers have been used as indicators of fluoride utilization including plasma, saliva, urine, nails, hair, teeth, bone etc. However, urine and plasma are the most commonly used biomarkers of fluoride exposure despite their downside as being indicative of exposure over a short period of time. These biomarkers have therefore been classified into contemporary, recent and historical markers. It is also pertinent to consider whether the biomarker of fluoride can be used on an individual or a population (Rugg-Gunn *et al*., 2011). Currently, urinary fluoride excretion is useful for predicting intake for populations but not for individuals (WHO, 2014).

2.4.1 Contemporary markers

Contemporary biomarkers determine the recent intake of fluoride and include blood, bone surface, saliva, milk, sweat, and urine. Since they correspond to the level of fluoride in different compartments of the body, the values are not a direct measure of fluoride accumulation in the body but indicate fluoride absorption (WHO, 1994).

2.4.1.1 Plasma

Plasma fluoride level establishes interstitial and intracellular fluoride concentration in soft tissues and it is dependent on total ingested fluoride dose, frequency of dose, the plasma half-life of fluoride (Rugg-Gunn *et al*., 2011; Cardoso *et al*., 2006) and physiological factors especially kidney function (Whitford, 1996; Cardoso *et al*., 2005). After ingestion of a small dose of fluoride which goes into the blood stream, plasma fluoride level increases within the first few minutes and reaches a peak in 20-60 minutes with a half-life of absorption of about 30 minutes (Rugg-Gunn *et al*., 2011). Fluoride concentration in plasma returns to its resting value in about 3 – 6 hours. Ekstrand *et al*. (1994) found a variation in attaining peak plasma fluoride between infants and adults and this was attributed to the longer time of fasting by the adults. In some of the infants, peak plasma concentration was not reached by 30 mins after the administration of fluoride tablet but 60 minutes, whereas in adults, peak plasma concentration was achieved by 30 mins. However, in this study, the peak plasma fluoride concentration was not correlated to the intake and this was attributed to the small range of fluoride intake and individual variability. There have been many reports on fasting (resting) plasma fluoride concentration in humans (Oliveby *et al*., 1989; Whitford, 1996; Maguire *et al*., 2005) which showed resting fluoride concentration ranging between 9.3 and 24.0 ng/ml for subjects living in an area with less than optimal fluoride concentration in water. It is essential to note that recent fluoride intake affects plasma fluoride concentration but sample of plasma collected in a fasting subject is not affected by recent exposure. Also, plasma clearance of fluoride dose varies with age (Ekstrand *et al*. 1980; 1994), with children utilising more fluoride for developing bones and this could affect the utilization of plasma fluoride concentration as a biomarker of exposure. In a study conducted among young children aged 4-5 and older children aged 12-14 years old (Ekstrand et al., 1980), peak fluoride concentrations between 60 – 120 ng F/ml were found within 2 hours, and after that plasma fluoride concentration rapidly decreased during the next 2 hours followed by a slower decrease. However, there were higher plasma fluoride concentrations in the younger

children (80 -120 ng F/ml) compared to the older children (60 – 80 ng F/ml) at the peak, even though the younger children were given a lower dose of supplement (3 mg F) compared to the older children (5 mg F). Perhaps surprisingly, at the end of 8 hours, plasma fluoride concentrations for both younger and older children were similar (Ekstrand et al., 1980). Therefore, resting plasma fluoride concentration, which seems not to be influenced by age, might be necessary when considering future biomonitoring programmes. Rugg-Gunn *et al*. (2011) showed a relationship between mean values of fluoride in plasma against the applied fluoride dose but the regression line obtained was not informative since the values were not from individuals. Determination of fluoride concentration in plasma involves collection of blood, which imposes difficulty in dental surveys (Schamschula *et al*., 1985) as the process is invasive. There have been insufficient data across age groups to determine the normal plasma concentration and hence deduce individual fluoride intake from plasma concentration. Table 2.1 reveals that studies conducted among children aged 2-10 years old with fluoride levels in drinking water in the range of 0.1-0.8 mg/l show resting plasma fluoride concentration between 16.9 and 24.0 ng/ml (0.89-1.26 μmol/l) and studies conducted in adults aged 19-56 years (drinking water fluoride 0.02-1.20 mg/l) show resting plasma fluoride concentration between 6.8 and 22 ng/ml (0.36-1.16 μmol/l). Most of these studies did not report the actual intake from diet and the use of dentifrice but other factors can affect plasma fluoride level such as age, altitude, genetic background, site of blood collection, haematocrit, hormones, effects of circadian rhythm (Rugg-Gunn *et al*., 2011). However, considering the study of Ekstrand et al (1980), age seems not to affect fluoride level in plasma when samples are collected in fasting subjects. This needs to be further investigated considering the importance of plasma in future biomonitoring studies.

2.4.1.2 Saliva

Saliva fluoride has been regarded as very important due to its strong influence on concentration of fluoride in plaque and dental caries control. Whole (or mixed) saliva has been used to assess caries prevention agents because it reflects the fluoride retained in the mouth from fluoridated oral hygiene products but it may not be a good biomarker due to such influence and contamination of fluoride from food. However, systemic saliva has been obtained by specially constructed devices from the parotid and submandibular/sublingual ducts, as it strongly correlates with plasma fluoride (Rugg-Gunn *et al*., 2011).

i) Whole saliva: Fluoride concentration in whole saliva is found to be increasing due to fluoride from food and the therapeutic agent associated with the prevention of caries. In a study by Hedman *et al*. (2006), the observed fluoride increase in stimulated whole saliva was attributed to the fluoride content of food debris. Toth *et al*. (2005) reported that fluoride level in whole saliva increased by about 10-fold with fluoridated milk (200 ml containing 5 mg F/l and 1.5% fats) and fluoride tablets (1.1 mg F/tablet) and about 6 to 7-fold for fluoridated salt (4 g containing 250 mg F/kg). Boros *et al*. (2001) showed that rinsing for 20-60s with fluoridated milk did not influence the fluoride level of unstimulated whole saliva 45 minutes later as well as in the control participants. This was contrary to an earlier report that fluoride released from food increased the whole saliva fluoride. Fukushima *et al*. (2011) also confirmed that whole saliva was less influenced by concentration of fluoride in water, as other dietary components consumed by the participant during the study might have increased the concentration of the whole saliva. Differences in these studies might be associated to the different sampling times since some showed influence of diets as well as therapeutic agents while others showed no effect. In this regard, whole saliva might be a useful biomarker of fluoride if employed correctly but it need to be investigated. However, some studies have investigated the effect of pH and salivary flow rate on the concentration of fluoride in saliva. Toth *et al* (2005) conducted a study among 20 healthy adults aged 19-45 years who ingested 1 mg fluoride daily in the morning as either fluoridated salt containing 250 mg F/kg, fluoridated milk containing 5 mg F/l or fluoride tablet containing 1.1 mg F/tablet (Dentocar, Hungary) showed increased fluoride concentration in saliva with no change in pH and salivary flow within two test periods separated by a two-week washout period. Other studies have also shown no significant change in flow rates when they were exposed to placebo and/or fluoridated dentifrice (Kavannagh *et al*., 1998; Boros *et al*., 2001; Hedman *et al*., 2006), although Hedman *et al*. (2006) attributed this to the limited number of participants. Duckworth and Jones (2015) showed an increase in flow rate but this was attributed to the presence of either sucrose or NaCl in the mouth rinses taken by the subjects.

In the study by Hedman *et al*. (2006) initial mean whole saliva fluoride values of 10.9 and 8.0 μg/L in the test and control legs, respectively, of 10 healthy adolescents fed specialised diets rose significantly to 81.6 μg/l in the test leg and to 31.5 μg/l in the control leg and remained elevated for 30 mins after ingestion with 50% increase in test diet between the control one. This was also in line with Bjornstrom *et al* (2004) who reported a prolonged elevation of saliva fluoride concentration of young adults after 30 minutes after consumption

of pop-corn prepared with fluoridated salt. Studies show that saliva fluoride concentration peaks rapidly between 1-15 minutes after eating food containing fluoride but, depending on the experimental conditions, it returns to baseline between 20-60 minutes (Patterson *et al*., 2002; Hofmann *et al*., 2003). Saliva might be a useful biomarker of fluoride exposure if samples are collected before participants consume food in the morning and prior to brushing their teeth or 2 hours after consumption of a meal or utilisation of any fluoridated oral hygiene product.

ii) Ductal saliva: Parotid ductal saliva has been reported to be related to plasma fluoride level and it has been very useful as a biomarker of fluoride showing the relationship between exposure to fluoride from water/food and retention in the body (Oliveby *et al*., 1989; Fukushima *et al*., 2011; Fukushima *et al*., 2006). Fukushima *et al*. (2011) in their study reported that the amount of fluoride in water was the main factor affecting the concentration of fluoride in parotid saliva and it is not directly affected by intraoral fluoride reservoirs, but majorly by systemic fluoride. However, age has been reported to affect the fluoride concentration in ductal saliva (Fukushima *et al*., 2006), but such was not seen in the whole saliva. Locality (urban/rural) has also been revealed to affect fluoride concentration in ductal saliva (Fukushima *et al*., 2011). However, several studies have shown a strong correlation between the fluoride level in parotid saliva and plasma (Ekstrand 1977; Oliveby *et al*., 1989; Whitford 1996; Whitford *et al*., 1999). It might be used to infer the body burden of fluoride.

2.4.1.3 Urine

Urine serves as the main pathway through which plasma fluoride and other extracellular fluids is eliminated from the body and excretion is proportional to the total fluoride intake (Whitford, 1996). However, several factors should be considered regarding the utilization of urine as a biomarker, including: urinary pH, glomerular filtration rate, person suffering from certain conditions e.g. kidney diseases; composition of diet. Because it is non-invasive, urinary fluoride excretion has a great advantage over other methods for monitoring community fluoridation schemes (WHO, 2014). Earlier studies, conducted when fluoride exposure was assessed mainly by the concentration of drinking water ingested, were of limited use, as they could not establish a correlation between fluoride exposure and urinary fluoride level (Schamschula *et al*., 1985; Czarnowski and Krechniak, 1990). However, more recent studies have been very useful since fluoride exposure was estimated by the amount of ingested fluoride from different sources including drinking water, foods, and beverages

as well as dental hygiene products. Urine can be referred to as a contemporary biomarker of fluoride, particularly when collected over a 24-hour period (Zohoori and Rugg-Gunn, 2000; Maguire *et al*., 2013; Hector *et al*., 2009; Haftenberger *et al*., 2001), since varying proportions of the fluoride taken in are excreted completely in urine below 24 hours in children and adults (Whitford, 1996). There also appear to difference in the proportion of fluoride ingested that is excreted in the urine due to age, usually higher mean urinary fluoride levels are observed in adults than in children (Czarnowski and Krechniak, 2002). Linear correlations were found for both children and adults between daily fluoride intake and daily urinary fluoride excretion, but the intercept and the slope for both age groups (0.15-7 years and 18-75 years) were significantly different and showed the higher percentage retention of total fluoride intake in children compared to adults, without any effect of gender (Villa *et al*., 2010). Conversely, Murray *et al*. (1991) showed that under stable fluoride exposure, urinary fluoride excretion was about 50% of the intake in adults and 30% in children. However, higher percentages between 51.5 and 85.0% of fluoride excreted in urine have also been revealed in children (Villa *et al*., 2000; Haftenberger *et al*., 2001). Urinary fluoride measurement is also influenced by the pH of the urine at the time of excretion (Whitford *et al*., 1994). There is an increase in urinary fluoride excretion, followed by a decrease in fluoride concentration of plasma, when urine is alkaline (WHO, 1994). This is in line with Ekstrand *et al*. (1978, 1980) who showed a lower renal clearance of fluoride in acidic urine compared to alkaline urine. This effect of pH on urinary fluoride excretion might principally be associated with the type of diet consumed, whether acidic or alkaline in nature. The type of diet determines the pH conditions in the gut and in the urine, the pH determines the form of fluoride in the gut, where acid yields HF that is more readily absorbed into the body than fluoride ions. Lakshimi and Lakshmaiah (1999) showed that excretion of fluoride in urine was significantly higher in rice based diets compared to shorgum based diets. Also, a randomised diet-control study among 18 boys in India, who received tamarind supplementation resulted in higher urinary pH and a subsequent increase in the urinary fluoride excretion (Khandere *et al*., 2002). Considering all these factors, it has been recommended as a biomarker of fluoride exposure in a community but not in individuals when fluoridation schemes are monitored (WHO 2014). Villa *et al*. (2010) showed 95% CI bands for plots of DUFE against TDFI and suggested that DUFE is not a precise estimator of TDFI on individual basis but suitable for predicting fluoride intake for population.

Table 2.1 Studies of plasma fluoride concentration in human subjects (adapted from Rugg-Gunn *et al*., 2011)

Author (year)	N	Age, years	Water fluoride (mg/l)	Resting fluoride	
				ng/ml	µmol/l
Ekstrand *et al*. (1977)	1	27	0.25*	10.3	0.54
Ekstrand (1978)	5	24-28	0.25	10.0	0.50
	5	27-56	1.20	20.0	1.00
	5	10-38	9.60	35.0	1.84
Ekstrand *et al*. (1981)	5	27-36	n.a.*	13.3	0.70
Oliveby *et al*. (1989a)	5	26-38	0.2*	9.3	0.49
Oliveby *et al*. (1989b)	5	26-38	0.2*	9.5	0.5
Oliveby *et al*. (1989c)	5	26-38	0.2*	12.4	0.65
Whitford (1996)	5	Adults	n.a.*	12.7	0.67
Whitford *et al*. (1999)	17	5-10	n.a.*	16.9	0.89
Levy *et al*. (2004)	15	2-6	0.6-0.8	19.0	1.00
	15	2-6	0.1-0.2	24.0	1.26
Maguire *et al*. (2005)	20	20-35	0.02*	19.8	1.04
Cardoso *et al* (2006)	5	25-35	0.03	9.7	0.51
	5		0.70	6.8	0.36[a]
	5		0.30	10.5	0.55[b]
Whitford *et al*. (2008)	5	24-32	0.85*	17.3	0.91
	5	24-34	0.85*	20.0	1.05
Cardoso *et al*. (2008)	5	27-33	Low fluoride	10.0	0.53

Author (year)	N	Age, years	Water fluoride (mg/l)	Resting fluoride	
				ng/ml	µmol/l
Buzalaf *et al*. (2008)	4	19-29[c]	0.6-0.8*	21.0	1.11
	4	19-29[d]	0.6-0.8*	22.0	1.16

n.a (not available)

*Subjects were exposed to a further fluoride dose

[a]Low fluoride intake from dentifrice

[b]High fluoride intake from dentifrice

[c]Fluoride administered as sodium fluoride after fasting blood samples

[d]Fluoride administered as disodium monofluorophospahte

Table 2.2 Studies on urine as a biomarker of fluoride

Age (years)	Age (years)	N	Urinary fluoride excretion (mg/d)	Source of fluoride intake	Fluoride content
Maheshwari *et al* (1981)	20-45[1]	20	0.53-0.98 3.14-4.12 5.78-7.47	Sodium fluoride supplements and meals	0.43 mg/d (Control) 5.40 mg/d (5 mg) 10.38 mg/d (10 mg)
Brunetti and Newbrun (1983)	3-4	10	0.28*		Optimal water fluoride
Ekstrand *et al* (1984)	0.19-0.54 0.15-0.31	5 BF 4 FF	0.030 0.359	Water	1.0 mg/l
Rugg-Gunn *et al* (1993)	4	44 53	0.420 0.550	Drinking water	1mg/l
Ekstrand *et al* (1994)	0.19-0.89	4 FF	0.144	Water	1.0 mg/l
Wang *et al* (1997)	5-6		0.33	Water	0.3 mg/l
Villa *et al* (1999)	3-5	42 46	0.229 0.526	Drinking water and F supplement (1mg in 50 ml orange juice)	0.57-0.62 mg/l
Villa *et al* (2000)	3-5	20	0.358	Drinking water	0.5-0.6 mg/l

Zohoori and Rugg-Gunn (2000)	4	78	0.339	Drinking water	0.30-0.39 mg/l
Ketley and Lennon (2000)	4-5	8	0.330	Milk fluoride	0.5mg
Baez *et al* (2000)	4-6	31	0.750	Drinking water	1.0-1.3 mg/l (school) 0.1-3.2 mg/l (Home)
Haftenberger *et al* (2001)	3-6	11	0.476	Fluoridated salt and tablets	0.25-1.0 mg/kg
Grijalva-Haro *et al* (2001)	8-9	11 10 11	0.930 1.040 3.100	Drinking water	0.54 mg/l 0.78 2.77
Villa *et al* (2002)	6-8	26	0.387	Milk	0.625 mg
Ketley *et al* (2004)	3	19 18 18 4 6 21	0.370 0.200 0.160 0.170 0.210 0.330	Drinking water	0.8-1.0 mg/l <0.15 <0.15 <0.15 <0.15 <0.15
Franco *et al* (2005)	4-5	96	0.414	Table salt	180-220 mg/kg

Buzalaf *et al* (2006)	20-35	10	0.952 - 1.642	Control (Diet and dentifrice) (Diet, dentifrice and fluoridated solution (1.8 mg NaF))	1.411 mg/d 1.626 3.426
Zohoori *et al* (2006)	1-3	7	0.33	Drinking water	0.81 mg/l
Maguire *et al* (2007)	6-7	18 8 3	0.203 0.239 0.323	Drinking water	0.08 mg/l 0.47 0.8
Acevedo *et al* (2007)	3-5	32 31	0.175 0.256	Drinking water, Salt Drinking water, Salt	0.12 mg/l, 60-90 mg/kg 0.34 mg/l, 60-90 mg/kg
Villa *et al* (2008)	20-40	60	1.24	Diet intake	1.82 mg/d
Zohoori *et al* (2012)	6-7	21 12	0.297 0.393	Drinking water	0.30 mg/l 1.06
Zohoori *et al* (2013)	≤4	29	0.530[a], 0.354[b] 0.092[a], 0.058[b]	Drinking water	0.6-0.8 mg/l <0.3
Rango *et al* (2017)	10-50	386	5.7[c]	Drinking water	6.4 mg/l

BF (breast fed), FF (formula fed), **including faeces*

[1]male

[2]female

Zohoori *et al* (2013a) urinary fluoride excretion when adjusted by body weight 0.039[a], 0.026[b] (0.6-0.8 mg/l); 0.008[a], 0.005[b] (<0.3 mg/l)

[a]Fluoride toothpaste user

[b]Fluoride toothpaste non-user

[C]12-hour urine period (overnight urine sample)

2.4.2 Recent markers

2.4.2.1 Nails

Fluoride concentration in fingernails, which is not usually affected by recent intake and other physiological factors, reflects average plasma concentration over a protracted period usually between 1-2 weeks (Amaral *et al*., 2014) but can reveal exposure for a period of 3-4 months depending on when the nail is clipped (Schamchula *et al*., 1985; Whitford *et al*., 1999; Czarnowski and Krechnaik, 1990; Whitford, 2005; Levey *et al*., 2004; Correa-Rodrigues *et al*., 2004; Buzalaf *et al*., 2006). Nails have also been used for monitoring occupational exposure to fluoride (Balazova 1971; Czarnowski and Krechniak, 1990; Susheela *et al*., 2013). Linhares *et al*. (2016) revealed that it could be used in epidemiological studies but their study showed a poor correlation to the intake which was established through the major water sources. It is also a good body burden fluoride biomarker from dentifrice. Amaral *et al*. (2014) revealed a 16% rise in fluoride concentration after 30 days but the lowest fluoride levels in nails were observed in approximately 3 months among children aged 18-30 months given 1100 μg F/g toothpaste. However, Lima-Arsati *et al*. (2010) showed variation in fluoride concentration in nails in the experimental periods of 35 weeks with no trend of decreasing after interruption of fluoride dentifrice among 1-3 years old children exposed to a combination of diet and dentifrice over a certain period. Whitford *et al*. (1999) showed that 3-6 mg/day of fluoride intake for a month reflected in the fluoride concentration in fingernail after 3.5 months and longer periods also for toenails. Other studies have shown that fingernails have a higher fluoride concentration than toenails (Whitford *et al*., 1999; Buzalaf *et al*., 2009; Amaral *et al*., 2014). This has been attributed to higher blood supply to fingernails than in toenails, extrinstic factors or analytical methods.

There is also possibility of fluoride incorporation from the environment. Czarnowski and Krechnaik (1990) attributed an increase in fingernail fluoride to external contamination. This was confirmed by Fukushima *et al*. (2008), who revealed that soaking nails in 1100 ppm fluoride dentifrice slurry for 3 minutes or in water with 100 ppm fluoride for 2 hours dramatically increased nail fluoride concentration, showing that it is possible for fluoride uptake from exogenous sources. Pessan and Buzalaf (2011) reported that toenails may be less susceptible to external contamination by fluoride than fingernails, and suggested the use of toenails (especially the big toenail) for future studies of evaluating nail fluoride levels. Alternatively, cleaning of nails either using diethyl ether or deionized water prior to analysis

would be useful in removing such external contamination. There has been concern that cleaning could result in a reduction of intrinsic nail fluoride concentration. However, Whitford *et al*. (1999) showed that prolonged immersion of nails in deionised or fluoridated water had no effect on the resulting fluoride concentration.

Considering the usefulness of nails as a biomarker of fluoride exposure, more studies on the sensitivity and specificity of the method are still needed before they can be validated. Pessan *et al*. (2005) evaluated the use of fingernails among 4-7 year olds with and without caries experience, given placebo and fluoridated dentifrice for a certain period. They found that nail concentration did not vary throughout the experiment and concluded that it lacks sensitivity to detect small differences in doses (Buzalaf *et al*., 2006; de Almeida *et al*., 2007). In contrast, nails showed high sensitivity (0.84) and moderate specificity (0.53) (Buzalaf *et al*., 2012) and therefore validated the use of nails as a predictor of dental fluorosis among children. There is still a need for more study to precisely determine the sensitivity of the method, i.e. the relationship between known, relatively small increments of fluoride intake and the associated increases in fingernail fluoride concentration.

Other factors, including concentration of fluoride in water, age, gender and geographic area, influence fluoride concentration of fingernails and toenails and should be considered during the utilization of these biomarker of fluoride exposure to predict dental fluorosis risk (Fukushima *et al*. 2009). Likewise, in interpreting the results of fluoride concentration in nails, nail growth rate as well as length needs to be considered. The available evidence strongly suggests that nearly all the fluoride in fingernails derives from the plasma through the growth end and not the nail bed (Whitford *et al*., 1999) and indicates that growth rate of nail and lengths are pertinent determinants when investigating sub-chronic fluoride exposure. Bannister (1995) showed that the growth rate is faster in younger than in older children and in the middle finger than in the little finger. Whitford *et al*. (1999) reported that children have shorter nails than adults. A study by Buzalaf *et al*. (2006) also reported that growth rate was statistically higher in the fingernail than toenail and that big toenails grew faster than the other toenails but such a contrast was not observed between the fingernails. More studies still need to be conducted on the digit difference in the rate of growth in nails.

Nails have shown to be promising among long and short-term markers of exposure to fluoride, as samples can easily be assessed and collected non-invasively, besides the storage possibility over a long period of time with the absence of degradation (Pessan and Buzalaf,

2011). Unlike urine, nail fluoride concentration is not influenced by differences in glomerular filtration rate, urinary pH and flow rate or dentifrice pH (Buzalaf *et al*., 2009).

2.4.2.2 Hair

Hair has been shown to be a good biomarker of fluoride for revealing long-term fluoride exposure (McKinney, 2000) and occupational exposures (Czarnowski *et al*., 1998), as it reflects the fluoride content of the metabolic environment during formation of the hair and is highly correlated with the drinking water fluoride concentration (Schamschula *et al*., 1985). Hair may be considered as a biomaterial for fluoride exposure biomonitoring to investigate children at risk of dental fluorosis regardless of the phase of tooth eruption (Mandinic *et al*., 2010; Antonijevic *et al*., 2015). The use of hair as a fluoride biomarker of exposure has been studied in humans and animals. A study conducted on rats with 20 ppm drinking water fluoride concentration or 8.7 mg HF/m^3 in the air for 2 hr/day revealed that more fluoride was stored in the hair, bones and teeth of the rats exposed to the airborne HF after 6 months than those exposed to the fluoride in their drinking water. Mandinic *et al*. (2010) reported high correlations of hair fluoride with dental fluorosis and water fluoride concentration among 12-year-old children. Also, Parimi *et al*. (2013) reported that low fluoride levels in water correlated with lower fluoride in hair and high fluoride in water correlated with higher fluoride in hair among 30 subjects (19 men and 11 women). These investigations have clearly revealed and confirmed the obvious value of fluoride hair analysis to determine body burden.

Kokot and Drzewiecki (2000) showed that lower levels of fluoride in water correlated with lower hair fluoride levels which ranged from 1.81µg/g to 2.32 µg/g but that differences in diet, tea drinking, and tooth brushing did not or did so only slightly among 548 samples of hair obtained from children and adults living in a low fluorosis risk region of Poland. Other factors like age, sex, medical conditions and hair care product use were not reported. The fear of external contamination, which might lead to overestimation of fluoride exposure, has discouraged its use as a fluoride exposure biomarker. Most toxicological laboratories have encouraged washing of hair prior to its analysis for certain elements of interest. But Kono (1997) showed a decrease in fluoride concentration to reference values after washing treatment in a follow-up study from the investigation of 142 male workers exposed to HF in Japan (Kono *et al*., 1990). Moreover, Stoarsky *et al*. (2000) revealed that unwashed hair may serve as a passive indicator of fluoride exposure particularly to gaseous fluoride compounds.

Hair has a huge advantage due to its ease of collection, storage (McKinney, 2000) and non-invasiveness. In addition, the effect of metals forming complexes with fluoride in hair is insignificant (Kokot and Drzewiecki, 2000). However, care should be taken with hair analysis because of the low intrinsic fluoride content as reported by Schamschula *et al*. (1985), who revealed that concentrations of fluoride in hair (0.18, 0.23, 0.40 ppm) were low, on average, compared to corresponding values in drinking water (0.09, 0.82, 1.91). In contrast, more recent studies have shown that there is a higher accumulation of fluoride in hair than in water. This might be associated with the method of analysis because the diffusion method of Taves (1965) used in these recent studies is able to remove the fluoride bound to the keratinized tissue of the hair, thereby making it easily detected by the ion selective electrode.

2.4.3 Historical markers

Bones and teeth have been referred to as biomarkers of the body burden of fluoride or historical markers, following the fact that fluoride is retained in mineralized tissue in humans as well as in animals (Rugg-Gunn *et al*., 2011).

2.4.3.1 Bone

Fluoride concentration in bones reflects cumulative exposure of fluoride uptake over a long period, but it requires a bone biopsy. Bone is usually difficult to obtain due to its invasiveness and, therefore, it is not applicable for large-scale studies or routine clinical situations (Vieira *et al*., 2005). The fluoride content of bone tends to increase throughout life because 99% of fluoride body burden is associated with calcified tissues, majorly with the skeleton (Whitford, 1994). Since fluoride concentration varies throughout the skeleton, there is a need for standardisation of the type/site of bone samples (usually iliac crest) (WHO, 1994; Vieira *et al*., 2005). Surface bone fluoride concentration may reflect contemporary fluoride intake while fluoride in mature bone was revealed to reflect chronic or historical fluoride intake (Pessan and Buszalaf, 2011). There are many factors that affect fluoride incorporation in bone. They include genetic background (Carvalho *et al*., 2009), renal function (Ekstrand and Spak, 1990), type of bone analysed (Eble *et al*., 1992), gender (Ishiguro *et al*., 1993) and increases with age.

2.4.3.2 Teeth

The challenges associated with bone collection and the invasiveness of the procedure has made dentine, one of the mineralized tissues in the teeth, an emerging historical marker as it also accumulates fluoride throughout life. Dentine does not undergo resorption and is easier to obtain than bone biopsies, particularly the commonly extracted third molar and premolars (Pessan and Buzalaf, 2011), while enamel (a second mineralized tissue of the teeth) shows the amount of fluoride taken up during tooth formation. Weatherell *et al*. (1972) associated the pattern distribution of fluoride in the enamel to pre- and post-eruptive uptake of fluoride. Fluoride concentration at an enamel depth of 2μm was 1700 mg/kg, 2200-3200 mg/kg, and 4800 mg/kg in areas where drinking water fluoride level was ≤0.1 mg/l, 1 mg/l and 5-7 mg/l, respectively (NRC, 1993). Schamschula *et al*. (1985) showed high fluoride content in enamel biopsies among 14 years old children drinking water with a fluoride concentration between 0.09 and 1.9 mg/l and the fluoride concentration was higher at the superficial surface than in deeper enamel biopsies with depths of 0.44-0.48 μm and 2.4-2.6 μm respectively. Enamel is affected by fluoride concentration only on its surface layer when the teeth have erupted. Despite its ability to predict the body burden of fluoride, various factors make enamel an unreliable biomarker including tooth wear (Weatherell *et al*., 1972), difficulty in utilizing equal sampling areas on contralateral teeth (Aasenden and Moreno, 2004), inconsistency from previous data (Richards *et al*., 1992; Vieira *et al*., 2004). Conversely, dentine has been regarded as the best marker to determine chronic fluoride intake and the most appropriate indicator of the body burden of total fluoride (Pessan and Buzalaf, 2011). Nevertheless, the normal level of fluoride in dentine is not yet established due to genetic variation and fluoride metabolism.

2.5 CORRELATION BETWEEN BIOMARKERS

Several studies have investigated the relationship between the different biological markers of exposure to fluoride. Maguire *et al*. (2007) found that urinary fluoride retention was not correlated with home water supply but was strongly positively correlated with TDFI. Fingernails and toenails were referred to as biomarkers of chronic exposure. Considering the difficulty and the expense of blood collection, an investigation conducted by Buzalaf *et al*. (2002) in rats showed a direct relationship (r = 0.67) between nail and plasma fluoride concentration. Levy *et al*. (2004) revealed a significant positive correlation (r = 0.57) between the mean of fingernail and toenail fluoride concentration and estimated fluoride

intake from diets among 2-6 years old children in a non-fluoridated community. Czarnowski and Krechniak (1990) showed a very strong positive relationship (r = 0.99) between fingernail and urine among 106 employees of a phosphate fertilizer plant, although lower between fingernail and urine was reported (r = 0.73) when individual values of subjects were compared. This study showed no correlation between the biomarkers and air of the phosphate fertilizer plant. Buzalaf *et al*. (2011) also showed a significant correlation between fluoride intake and fluoride excretion in urine as well as fluoride concentration in fingernails and toenails among 4-6 years old children in Brazil but the correlation was lower in urine (r = 0.28) compared to those observed for fingernails and toenails (r = 0.36). This might be associated with factors affecting urinary fluoride excretion mentioned earlier. However, in this study, 24-hour urine was not validated to ensure that only complete 24-hour samples were included and analysed. Villa *et al*. (2000) showed a slight relationship (r = 0.513) between fractional urinary fluoride excretion (FUFE) and the inverse of daily fluoride dose (1/dose) but they found a very strong significant correlation (r = 0.98) was observed when previous studies on fluoride retention was combined with their present data on FUFE and urinary excretion. There is a need for further research on these biomarkers, particularly those that have not been fully investigated for their benefits to be fully utilised in identifying children at risk of dental caries as well as dental fluorosis and to provide a basis for decisions on fluoride use for dental caries prevention.

Table 2.3 Fluoride concentration in nails (modified from Pessan and Buzalaf, 2011)

Author (year)	N	Age range, years	Fluoride source (ppm)	Fluoride concentration	
				Fingernails (μg/g)	Toenails (μg/g)
Schamschula *et al*. (1985)	139	Children	< 0.1 0.5-1.1 1.6-3.1 [a]	0.79 1.31 2.31	
Czarnowski and Krechniak (1990)	110	21-61	Work [b] Control	19.2-624 8.4	
Schmidt and Leuschke (1990)	38	42-86	Air [c] Control	0.46 0.79	
Spate *et al*. (1994)	25	Adult *	0.1 [a] 1.0		4.2 6.4
Whitford *et al*. (1999)	46	6-7	0.1 [a] 1.6 2.3	1.85 5.28 7.52	
Correa Rodrigues *et al*. (2004)	10	2-3	Fluoride-free 1579ppm [d]	2.7 5.0	
Levy *et al*. (2004)	30	2-6	0.1 [a] 0.8	2.3 3.6	1.6 2.8
de Almeida *et al*. (2007)	33	1-3	0.130 [e]	2.22-3.53	
Buzalaf *et al*. (2009)	60	5-6	1100 (pH 7) [d] 1100 (pH 4.5)	2.89 2.74	2.42 2.25

Author (year)	N	Age range, years	Fluoride source (ppm)	Fluoride concentration	
				Fingernails (µg/g)	Toenails (µg/g)
			550 (pH 4.5)	1.75	1.49
			1100 (pH 7)	3.43	2.80
Lima-Arsati *et al*. (2010)	23	1-3	0.072-0.098 [f]	1.96-3.18	
			0.036-0.043 [e]	2.76-3.91	
Buzalaf *et al* (2011)	121	4-6	AFW	2.11	1.42
			NFW	3.38	2.24
			FS	6.09	6.70
			FM	2.62	2.63
			No F source	1.93	1.62
Amaral *et al*. (2014)	56	18-30 months	0.025 [f]	2.44	2.19
			0.027	2.66	2.15
			0.040	3.26	2.64
Sankhala *et al*. (2014)	40	>20	4.1 [a]		82.38
			4.8		86.14
			5.6		103.92
Linhares *et al*. (2016)	66	4-12	0.77 [e]	2.47	
			0.24	1.27	
			0.30	1.14	
			1.20	2.78	

Author (year)	N	Age range, years	Fluoride source (ppm)	Fluoride concentration	
				Fingernails (µg/g)	Toenails (µg/g)
	63*	25-50	0.93 [e] 0.33 0.51 1.81	1.40 0.67 1.43 2.75	
Rango *et al*. (2017)	386	10-50	6.4 [a]	5.3	

(AFW, artificial fluoridated water; NFW, natural fluoridated water; FM, fluoridated milk; FS, fluoridated salt)

**women participants, a (drinking water (mg/l)), b (work exposure), c (air exposure), d (toothpaste fluoride (ppm)), e (diet and dentifrice (mg/kgbw/d)), f (fluoride in diet (mg/d)).*

2.6 ANALYTICAL METHODS FOR FLUORIDE ANALYSIS OF BIOMARKERS

Fluoride in biological tissues and fluids including plasma, saliva, urine, hair, nails etc. can be present in its organic form covalently bound within an organic molecule or in inorganic form. The organic form of fluoride is neither diffusible nor absorbable whereas inorganic fluoride is both diffusible and absorbable and can be present as either ionic or nonionic bound to hydrogen as HF, complexes with cations e.g. Fe^{3+}, Ca^{3+}, Mg^{2+}, Al^{3+} etc. or salivary material and plasma proteins (Venkateswarlu, 1990). The ionic fluoride is readily available while the inorganic nonionic fluoride can be converted to the ionic fluoride by an unmasking agent. There are different analytical methods that can be used for identifying, quantifying and monitoring of fluoride in the various biological markers depending on the form in which the fluoride is present e.g. ion selective electrode, volumetric method, ion chromatography, radiochemical method, colorimetric method, gas chromatographic method and non-destructive nuclear methods (Charles, 1983). However, several factors need to be considered when using these analytical methods, including: separation of the fluoride from interfering

substances, the employed method should give a low blank value, the final fluoride concentration should be adequate for measurement by the analytical method (Omid *et al*., 2013).

2.6.1 Pre-treatment of samples

Biological samples such as urine, blood, saliva, and other bodily fluids can be analysed with minimum preparation. However, tissues such as nail and hair require a certain level of preparation to free fluoride from its matrix. Pre-treatment is necessary to isolate the ionic, complex and covalently bound fluoride as well as remove organic matter that can interfere with the fluoride analysis (Omid *et al*., 2013). The most commonly employed methods are: open ashing at a temperature of about 600^{o}C (Singer and Ophaug, 1986; Boivin *et al*., 1988), fusion with alkali (Baker, 1972), oxygen combustion (Haldinmann & Zimmerli, 1993) and acid digestion (Taves, 1983). Open ashing is unsuitable for small samples due to physical loss of low density ashes and loss by volatilization as well as contamination from the muffle furnace (Hall, 1968). It is also time consuming (Malde *et al*., 2001) compared to, for example, acid digestion which has the advantage of completion within minutes with no fluoride loss or contamination from extraneous sources (Grobler *et al*., 1998). Acid digestion does not liberate fluoride bound strongly within silicate materials (Stevens *et al*., 1995). The use of fusion separation to separate interfering substances requires very precise control to obtain reliable results and it is time consuming (Wade and Yamamura, 1965; Singer and Armstrong, 1965).

2.6.2 Separation and concentration of fluoride ions

Distillation of fluoride, a method developed by Willard and Winter (1933), had been used for the separation of fluoride by the volatilization of hexafluorosilicic acid with vapour from perchloric or sulphuric acid at 135^{o}C in the presence of glass beads or powder. It has since undergone several modifications such as the use of temperature-controlling devices and compressed air to replace the steam or distilled water normally used to drive over the fluoride or vacuum. Also, substitution of perchloric or sulphuric acid with ammonium bisulphate, using an aspirator-induced air stream. Despite the improvements, the method requires elaborate apparatus and the final concentration of the sample is reduced (Bellark, 1957). A micro-diffusion technique modified by Taves (1968), which allows fluoride to be released from both organic and inorganic matrices through overnight diffusion (between 16-24 hours) with acid-hexamethldisiloxane, is commonly used lately due to its advantage over

other methods like distillation, pyrolysis and spectrophotometric techniques (Winters, 1971). However, care must be taken by the analyst to prevent sample contamination, incomplete release of fluoride from matrices and loss by volatilisation when preparing the sample (NRC Canada, 1971). Fluoride in fingernails, for example, has been quantitatively removed using this method of preparation (Whitford *et al*., 1999).

Other techniques include anion exchange resins in acetate, hydroxyl and chloride form for separating fluoride in soft tissues, bone, urine and water (Nielson and Dangerfield, 1955; Kelso *et al*., 1964); reverse extraction of fluoride for separating interfering substances (Venkateswarlu, 1974) and adsorption of fluoride using magnesium oxide or calcium phosphate (Venkateswarlu and Narayanarao 1953). Table 2.4 below shows the preparation method and analytical methods used for the different biomarkers. Most of the challenges associated with these preparation techniques have previously been discussed in section 2.4.

Table 2.4 Analytical method of fluoride analysis in biomarkers (modified from Tylenda, 2011)

Sample	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Extract with TMCS (trimethylchlorosilane); inject organic phase (microwave induced plasma emission detector)	GC	4 µg/l	93.5%	Chiba *et al*. 1982
	Add equal volume TISAB (Total ionic strength adjustment buffer) solution	ISE, NIOSH 8308	0.1 mg/l	95%	NIOSH 1994
	Add TMCS toluene solution; centrifuge; inject toluene layer	GC	>5 ng/ml	No data	Ikenishi *et al*. 1988
Saliva	Mix in TISAB buffer; analyse	ISE	No data	99.8%	Petersson *et al*. 1987; Schamschula *et al*. 1985
	HMDS (Hexamethyldisiloxane)-facilitated diffusion overnight	ISE	No data	No data	Whitford *et al*. 1999
	Centrifugation and filtration	ISE			Van Loveren *et al*. 2004

Sample	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
	Mixed with TISAB; centrifuged; analyse clear supernatant	ISE	No data	No data	Hedman *et al*. 2006
Biological fluids and tissue extracts	Absorb with calcium phosphate; centrifuge; analyse	ISE	10 µg/l	92-102%	Venkateswarlu *et al*. 1971
Biological materials	Extraction form acidified sample as fluorosilane; reverse extraction as fluoride ion into alkaline solution	ISE with hanging top assembly	>0.04 ng/sample	No data	Venkateswarlu 1974
Biological fluids	Add TMCS toluene solution; centrifuge; inject toluene layer and analyse by measuring TMFS (trimethylfluorosilane) peak height	GC	5 ng/l	88.1-97.2%	Ikenishi *et al*. 1988
Biological tissues	Sample pulverized to fine powder; irradiate with energetic beam of protons; detect Gamma rays emitted	Proton Activation Analysis	<10 ng/sample	No data	Rudolph *et al*. 1973
	decomposition of sample at 700-1000^{o}C (pyro hydrolytic technique)	Colorimetry	1 µg/sample	No data	Kakabadse *et al*. 1971
Plasma	HMDS-facilitated diffusion overnight	ISE	No data	>99%	Carvalho *et al*. 2006

Sample	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Hair	Washed in distilled water and acetone	XRF spectrometer	1ppm	No data	Kono *et al*. 1990
	Wash in diethylether; dry; de-compose in NaOH	ISE	No data	94-96%	Schamschula *et al*. 1985
	Rinsed with acetone, detergent 2N sulphuric acid and redistilled water; dry; treated with NaOH solution; heated in boiling water to solution; neutralized with 1M HCl; add TISAB	ISE	No data	100± 8%	Stolarska *et al*. 2000
	Washed with acetone; with 0.5M H_2SO4; with distilled water; fluoride isolation by Obrink method (microdiffusion)	ISE	No data	No data	Kokot and Drzewiecki 2000
	Mineralised with 1.5ml 40% AgClO4 and 1.5ml 70% HClO4; mix with TISAB; neutralized with 5 mol/l NaOH	ISE	0.02ppm	93%	Mandinic *et al*. 2010
Fingernail	HMDS-facilitated diffusion overnight	ISE	No data	No data	Whitford *et al*. 1999
	Wash in diethylether; dry; de-compose in NaOH	ISE	No data	94-96%	Schamschula *et al*. 1985

GC, Gas Chromatography;

ISE, Ion Selective Electrode; NIOSH, National Institute for Occupational Safety and Health,

2.6.3 Measurement of fluoride

Several methods have been used to quantitatively measure fluoride in biological markers but the most commonly used is the potentiometric method (fluoride ion selective electrode) which has also been recommended for the measurement of fluoride in urine by NIOSH (Method 8308) (NIOSH, 1994). The ion selective electrode (ISE) has a membrane made up of lanthanum fluoride crystal doped with europium (II) for conductivity improvement (Skoog *et al*., 1990). The ISE is very sensitive, rapid and simple with recoveries greater than 90% depending the sample preparation required and type of sample. The limitation of the use of fluoride ISE is associated with the interference from hydroxide ion but this can be corrected by adjusting the pH of the solution to approximately 5 by addition of total-ionic strength adjustment buffer to the standard and sample (Tylenda, 2011). It cannot be used to measure monofluorophosphate and organic fluoride directly but after treatment of the sample by the HMDS diffusion method, the fluoride is released and can be detected by the ISE. This method has been extensively used for measurement of fluoride in biological media and has also been recommended by WHO for monitoring of fluoride in urine (WHO, 1999).

Gas chromatography (GC) has been used to measure fluoride in some biological media including urine and plasma (Ikenishi and Kitagawa 1988; Ikenishi *et al*., 1988) due to its high sensitivity to detect nano-gram quantities of fluoride. GC may also be affected by interference from aluminum ion as it does with ISE. Other methods have been developed, including: calorimetric methods which are time consuming (Quentin, 1967); fluorometric, neutron activation analysis (Knight *et al*., 1977), aluminum monofluoride molecular absorption spectrometry (Tsunoda *et al*., 1977; Venkateswarlu, 1992) and enzymatic methods which are sensitive to measuring fluoride in trace amounts of samples but the equipment required is quite expensive.

2.7 ACCEPTABILITY OF THE USE OF BIOMARKERS AND ETHICAL ISSUES

The utilization of biological markers of exposure has made measuring the bioavailable fluoride possible, thereby making it possible to identify populations at risk of dental caries as well as dental fluorosis. Biomarkers help to prevent the over estimation or under estimation of exposure to fluoride associated with dietary intake methods. The collection and use of biological samples in research still generates ethical issues e.g. confidentiality and ownership etc. and policy issues (Ashburn *et al*. 2000; Bauer *et al*. 2004). Interestingly, an inquiry which dealt with postmortem organ and tissues retained from children for medical

research conducted by Alder Hey and Bristol Royal Infirmary attracted some negative publicity (Burton and Wells, 2001). Several studies have since been conducted in some countries on participants' preferences for the collection and storage of biological markers, including: Scotland (Haddow *et al*. 2007), the United Kingdom (Treweek *et al*. 2009), Canada (Willison *et al*. 2007), the Netherlands (Vermeulen *et al*. 2009), Sweden (Johnsson *et al*. 2008), the United States (Wendler 2006), Asia (Zhang *et al*. 2010), Uganda (Wendler *et al*. 2005) and Egypt (Abou-Zeid *et al*. 2010). American and European studies have reported relatively high public willingness to contribute biological samples for research (Hoeyer *et al*. 2004; Kettis-Lindbland *et al*. 2005; Pentz *et al*. 2006). A study conducted among 2000 subjects in Peterborough showed that 99% were in favour of provision of tissue from living subjects for research (Royal College of Pathologist, 2002) but this does not reflect public opinion in general. A study conducted in a Newcastle NHS dental practice showed that 18% of the participants would not give consent for research to be carried out on their tissue and 50% would not give consent for their child's tissue donation (Goodson and Vernon, 2004). Some cases of abuse and misuse of participants' biological samples have been reported where biosamples were used for research not specified in the consent form (Mello and Wolf, 2010; Vorhaus, 2010), full consent was not given (Kleiner, 2000) or the consent was not clear if participants provided samples for the project described or a future project (Schmidt, 2001). More still needs to be done to safeguard and reassure the public against malpractices (Goodson and Vernon, 2004) as well as understanding public preferences regarding the use of biological samples. Abou-Zeid *et al*. (2010) showed that 80% of the participants would participate in a study involving collection of blood and storage for future research but 40% wanted options that the research should be restricted to the current disease studied and an option for unlimited use whereas 80% of participants in a malaria study conducted in Uganda did not find any future concern about the use of their biological samples as long as the research was approved by an institutional review board (Wendler *et al*. 2005). In another study, about 50% of the participants preferred to be contacted prior to future use of their samples as well as other issues of concern including benefit sharing, export of samples, ownership rights (Moodley *et al*. 2014). Fillipi *et al* (2012) also showed 70.2% would be willing to provide saliva for a specific study with proper consent in a study conducted among American Indian/Alaska Natives. Therefore, understanding how people feel about participation in studies is crucial for community involvement in research involving the collection of biological samples; participants must be

guaranteed proper use of samples and consent must be written to favour both parties and in clear language.

There might also be intercultural and intercountry differences associated to the differences in perception and familiarity of health research between developed and developing countries. There has been a limited amount of research conducted in developing countries in this regard, particularly in Africa with its vast cultural, religious and geographical diversities (Wendler *et al*. 2005; Abou-Zeid *et al*., 2010; Van Schalkwyk *et al*., 2012; Igbe and Adebamowo, 2012; Tindana *et al*., 2012; Moodley *et al*. 2014).

Despite the benefits associated with the use of biomarkers of fluoride exposure, there have not been any published data on the perception of the subjects about its utilization as they might find it difficult providing the sample for analysis considering the potential problems associated with its collection. Collection of blood is invasive, requiring a needle penetrating the skin, which sometimes might be painful and some parents might find it difficult to see the discomfort of their children during blood collection. On some occasions, children might develop fear on visits to general practice. Researchers investigated other biomarkers due to some of these limitations. For example, 24-hour urine collection, which is also a short-term measure and does not require an invasive procedure, still might pose certain difficulties as subjects need to carry the equipment for collection for the 24-hour period which might not be convenient or they will have to stay at home during collection and this might deny then of a day's pay. Children might be difficult to monitor during the collection period except when properly supervised by their teacher. Also, in developing countries, where most children aged 3-5 years living in rural areas don't go to school and do not have good toilet habits or there is no toilet, parents might find it difficult supervising such collection.

Recent markers, e.g. nails and hair, which are useful for long-term exposure to fluoride and collection is non-invasive, are not without their challenges associated with how they are collected due to peoples' preferences for maintaining them. Some people prefer to keep long nails and hair or might not want to discontinue the use of certain cosmetic products, particularly females. However, Sankhala *et al*. (2014) revealed that all donors easily consent to the use of toenail. The attitude towards a biomarker may vary according to age, gender, religion, academic level and discipline, socio-economic factors as well as culture. However, these factors remain uninvestigated while biomarkers of fluoride are becoming popular in epidemiological studies. A study conducted by Omid (2011) among 4-6 years old children

living in north-east of England investigated the level of compliance of parents who were asked to provide 24-hour urine samples. This work showed that only 7.7% of the parents consented to participate in the study and 1.5% of those who consented dropped out prior to completion of the study despite the incentive provided. There is a need for further research on people's perception regarding each of these biomarkers following their utilization in monitoring exposure to fluoride.

2.8 STATUS OF FLUORIDE ON HEALTH IN NIGERIA

Analysis of fluoride levels in water taken from different parts of Nigeria has variable fluoride concentration. Fluoride level in samples of water in western Nigeria varied from 0 - 0.4 mg/l and in Northern Nigeria from 0 – 1.2 mg/l (El-Nadeef and Honkala, 1998) but other studies have reported drinking water fluoride up to 10.3 mg/l in North Central, Nigeria (Akpata *et al*., 2009; Dibal *et al*., 2012). The distribution of fluoride in different parts of Nigeria is presented in Figure 2.1 (Akpata *et al*., 2009). Akpata *et al*. (2009) also showed fluoride concentrations from different water sources collected from different geopolitical zones of Nigeria, which are presented in Table 2.4. The study showed that 58-70% of Local Government Areas (LGA) had drinking water sources that contained 0.3 mg/l fluoride or less, except for the Northern Central geopolitical zone where only 26% of the LGAs had water sources containing the low fluoride (Akpata *et al*., 2009). Fluoride level within the Northern Central geographical area varied widely, as some water sources (particularly wells and boreholes) show significantly higher fluoride levels. Makurdi, the capital of Benue state located in the North Central region, had high fluoride concentration (6.7 mg/l) recorded in a deep well and fluoride concentrations above 1.5 mg/l were reported in Langtang, Isoko North, Esan West, Ilejemese (Akpata *et al*., 2009) and Kaltungo town (4 mg/l) in Gombe state (Dibal and Lar, 2005). A fluoride content in drinking water of 8 mg/l was recorded in Dorong and Furzi villages in Jos east local Government Area of Nigeria (Lar *et al*., 2007). Thirty-two percent of water sources analyzed in the Langtang LGA of Plateau state were within the WHO recommended range of 0.5-1.5 mg/l, 12 % were lower while 56% were higher than 1.5mg/l (Dibal *et al*., 2012).

Studies show that the concentration of fluoride in water was dependent on the source of water. Waziri *et al*. (2012) revealed that fluoride concentration in surface water in Northeastern Nigeria ranges between 0.03 mg/L and 2.07 mg/l, while groundwater ranges between 0.02 mg/l and 2.42 mg/l, with 43% of sampling points showing fluoride

concentrations higher than 1.5 mg/l recommended for fluoride in groundwater by the World Health Organization (WHO). Studies on the prevalence of dental fluorosis have been conducted in some parts of northern Nigeria due to high levels of fluoride in drinking water identified in some of the areas and several cases of dental fluorosis have been reported in Bauchi, Gombe, Adamawa, Plateau, and Benue (Dibal *et al*., 2012). However, it has also been revealed that some areas (Plateau and Bauchi), where water fluoride ranged from 0.0-0.4 mg/l, showed prevalence of dental fluorosis in 51% of children aged 12-15: 41% had very mild fluorosis, 7% had mild fluorosis and 3% had moderate to severe fluorosis (El-Nadeef and Hankala, 1998**).** It was also observed in all study areas in central Nigeria, where fluoride levels were less than 0.5 mg/L, that majority of caries-free children had a very mild form of fluorosis (El-Nadeef and Honkala, 1998). It seems unlikely that water consumption by these children would be the only explanation for fluorosis due to fluoride exposure from drinking water was very low. However, other sources of fluoride were not investigated. Guskit (2010) also observed that cases of dental fluorosis are common in inhabitants of Payan area, Jos, Nigeria especially in the age group between 40-50 years exposed to <0.5 mg/L fluoride and some cases have also been reported in the Jos east LGA (Jaryum, 2005). Akosu and Zoakah (2008) revealed 12.1% of dental fluorosis in the same area (Central Plateau Senatorial District) where fluoride concentration is generally low (<0.5 mg/l), but with higher dental fluorosis prevalence (21.9%) in area with high altitude (mean height above sea level = 1700 m) compared to a low altitude area (mean height above sea level = 1000 m) where subjects showed 3.5% dental fluorosis. However, because of stratified analysis of control for the confounding effect of altitude, fluoride prevalence is not significantly associated with the fluoride level of water in the high-altitude area (0.68 (0.004) ppm) which account for most of the fluorosis in the district. This suggests that other risk factors apart from fluoride exposure from water may be responsible for the occurrence of dental fluorosis in the area.

Clear manifestations of dental fluorosis and bowing of legs, especially in children between the ages 7-11 years, have been reported in the inhabitants of Dorong and Langtang located in Plateau state of Nigeria where fluoride concentration of 7.0-10.3 mg/L is reported in water which is five times more than the 1.5mg/L WHO recommended limit (Dibal *et al*., 2012a; Dibal *et al*., 2012). Wongdem *et al*. (2007) also revealed dental fluorosis was found to be 26.1% in children between ages 10-19 years in the same area. However, some children and adults, although born and raised in these areas with high fluoride concentration in drinking

water, do not show any evidence of dental fluorosis. Such individuals may be protected by genetic predisposition or by environmental factors (Dibal *et al*., 2008).

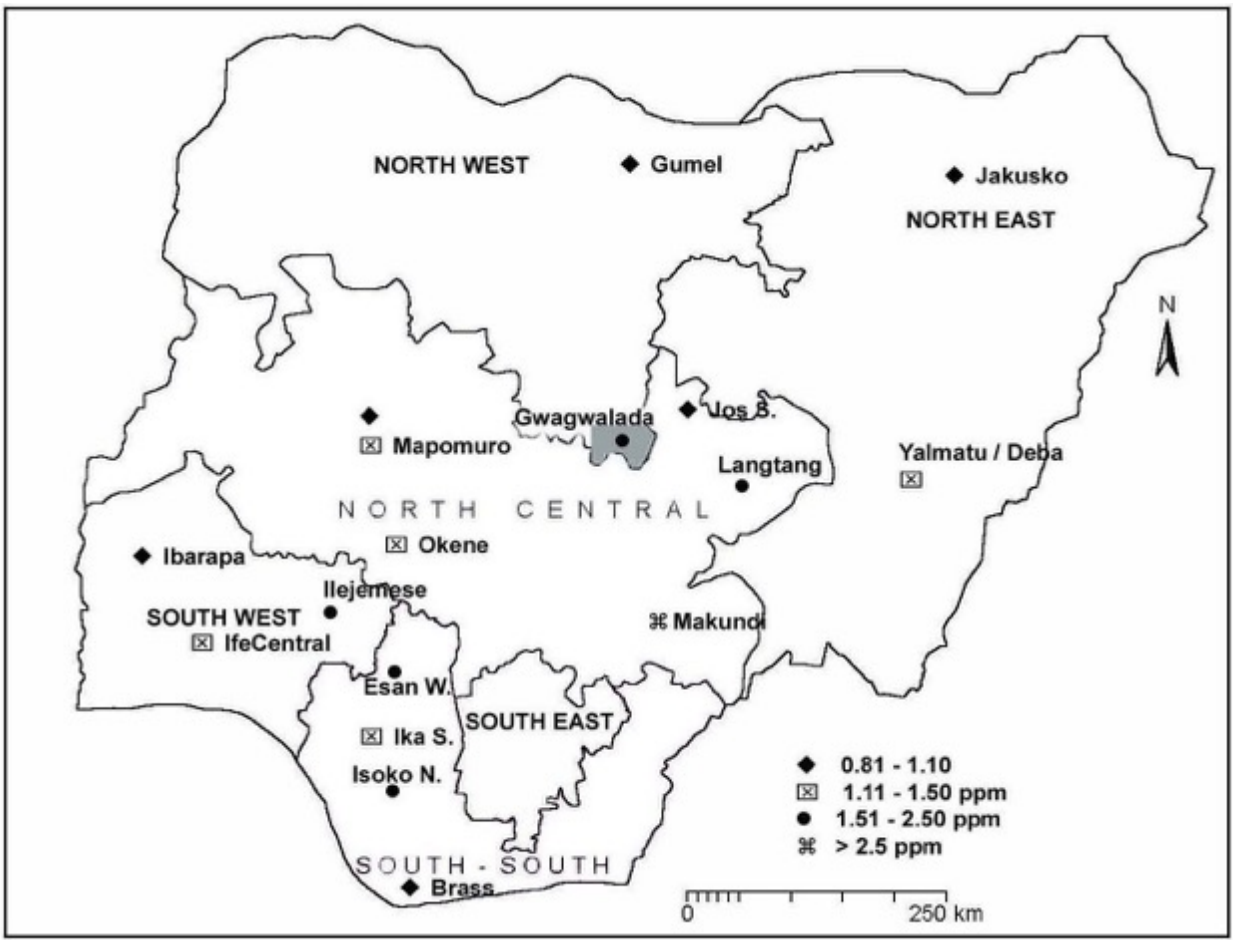

Figure 2.1 Map of Nigeria showing Local Government areas with drinking water containing fluoride levels higher than 0.8 ppm in the six geopolitical zones (Akpata *et al*., 2009)

Table 2.5 Mean fluoride level (±SD) in various drinking water sources in Nigeria (Akpata *et al*., 2009)

Geopolitical zone	Drinking water source						
	Water-works	Rivers/Streams	Shallow wells	Deep wells	Boreholes	Ponds	Others
N. Central+FCT	0.70±0.62	0.41±0.31	0.96±0.81	0.67±1.28	0.68±0.75	0.44±0.24	0.36±0.23
N. East	0.55±0.46	0.28±0.28	0.33±0.22	0.36±0.30	0.62±0.67	0.38±0.09	0.11±0.05
N. West	0.32±0.29	0.31±0.16	0.26±0.22	0.33±0.28	0.34±0.24	0.31±0.21	0.01±0.00
S. South	0.22±0.12	0.22±0.34	0.28±0.76	0.24±0.38	0.53±1.10	0.10±0.03	0.22±0.26
S. East	0.44±0.66	0.23±0.20	0.21±0.08	0.18±0.05	0.38±0.42	0.32±0.37	0.31±0.37
S. West	0.16±0.10	0.17±0.11	0.26±0.19	0.33±0.35	0.63±1.05	1.72±3.07	0.17±0.15
Total	0.39±0.44	0.27±0.26	0.39±0.60	0.40±0.67	0.50±0.75	0.50±1.16	0.24±0.28

FCT (Federal Capital Territory

2.9 SIGNIFICANCE OF STUDY

Fluoride has been shown to be very beneficial in the prevention of dental caries. However, excessive exposure to fluoride in drinking water as well as other sources including diets and oral hygiene products could lead to dental fluorosis. Fluoride ingested during dental development until the age of six years may promote the development of fluorosis depending on the dose and duration of exposure. Monitoring of fluoride has therefore been recommended for the benefit to be fully maximized, particularly when fluoridation programmes are considered or are in place. Several studies have investigated exposure to fluoride principally by monitoring fluoride in drinking water. However, it is essential to also note that fluoride in drinking water is not the only source of exposure to fluoride, other sources of fluoride exposure are increasingly becoming more significant e.g. beverages, dentifrice etc. Geographical movement of certain foods from regions of production where fluoride in drinking water is high to areas where water fluoride is low (halo effect) have changed the scope of exposure. Other studies have utilized several diet assessment techniques in monitoring the intake of fluoride from the diet and intake from dentifrices has been quantified by either measuring fluoride in expectorated saliva or through semi-quantitative questionnaires; but these methods are not without their challenges. Studies of dental fluorosis and dental caries are therefore difficult to interpret and some of the challenges have been suggested to include the fact that: few studies reported an interpretable range of exposure to fluoride; some results tended to be contradictory, with no clear-cut trend in both men and women as well as children and adults; total fluoride intake was not estimated; and for some studies total intake of fluoride is difficult to estimate, which could be attributed to the method used for quantification or the halo effect described above.

Biological markers are primarily used for identifying and monitoring deficient and excessive intakes of biologically available fluoride (which is the amount of ingested fluoride that is absorbed and potentially responsible for disturbing amelogenesis). They have been investigated for monitoring exposure to fluoride and for assessing the impact of water fluoridation on bone quality and other physiological conditions (Mullane *et al*., 2016). Fluoride concentrations in bodily fluids (e.g., urine, plasma, serum, saliva) are probably most suitable contemporary markers for evaluating recent or current fluoride exposures or fluoride balance (intake minus excretion) and are used for acute exposures due to rapid

elimination of fluoride and measurements can be done shortly after samples are taken. In contrast, hair and nails (fingernail and toenails) are useful for measuring exposure over a long period of time. Extensive studies have been conducted on the biomarkers of contemporary and recent fluoride exposure and a summary of the research has been published (Rugg-Gunn *et al*., 2011) but the conclusion that these tissues are suitable indicators of fluoride utilization and accumulation in the body is premature. Biological tissues or fluids have varying degrees of accuracy, timed collection of blood plasma and parotid saliva have been used to monitor the time course of body fluid concentration of fluoride following ingestion of fluoride tablets, likewise the analysis of 24-hour urinary fluoride concentrations which is the most frequently used biomarker has provided useful information particularly on percentages of fluoride retention in children and adults. Among the recent markers, nails appear to be more studied compared to hair samples for estimating exposure over a long period of time but both have been used in investigating the body burden of fluoride.

Presently, these biomarkers have not been fully investigated; there are many factors that are affecting the utilization of each of them. The data obtained for urine, when it is collected over a 24-hour period to measure fluoride exposure, are less precise and more difficult to interpret compared to analysis of plasma. The use of 24-hour urine has not been without challenges associated with urinary flow and pH which will influence the fluoride output. It is also reported that past fluoride exposure is also a factor that influences the urinary flow output due to the large fraction of fluoride accumulated in bone that is slowly released (SCHR, 2010). Dietary practices have also been considered as one of the factors known to influence urinary fluoride excretion (Mullane *et al*., 2016). There are insufficient data on plasma fluoride concentrations across various age groups to determine the usual fluoride concentration as well in saliva which is also difficult to interpret due to contradicting information regarding its contamination by diets or therapeutic agents. There are also inconsistencies in data obtained by different studies on the use of hair and nails as biomarkers of exposure, some associated with methodological issues others with its practicality. Their use as a non-invasive biomarker of chronic/sub-chronic fluoride exposure has not been fully investigated and few published data are currently available, particularly for hair. Fluoride in blood, urine, saliva, nail and hair samples has not been investigated systematically with respect to their use as an exposure biomarker. The only data available utilized water as the indicator of fluoride exposure (Schamschula *et al*., 1985). The present

data are also insufficient using fluoride concentration in these tissues and fluids as biomarkers of contemporary and recent fluoride exposure to individuals.

There is a need for additional research to find the best marker across various age groups to determine the normal concentrations of fluoride in these biomarkers for populations exposed to very low and endemic fluoride areas based on several factors, including: feasibility of measuring the marker, invasiveness of sampling technique, amount of specimen needed, stability of the marker, time to appearance of the marker and finally cost, sensitivity, specificity and reliability of each assay as well as acceptability of the markers. Considering the ability of these biomarkers to assess exposure to fluoride, at present, the most reliable biomarkers are not yet known. Therefore, to have a more precise indicator of fluoride levels in humans, particularly children at risk of developing dental caries as well as dental fluorosis, a search for the best biomarker of fluoride exposure needs to be conducted, comparing the different biological markers and considering the issues raised. This would be particularly useful because of the complexity of biomarker chemistry and environmental exposure to fluoride and associated illnesses as well as social problems involved. If biological markers are not carefully studied and their usefulness identified at both the individual level and in a group, it is also possible to measure the wrong biomarker, obtain accurate but inappropriate information and draw a false and misleading conclusion.

This research will, therefore, investigate comparing both contemporary (e.g. blood, saliva and urine) and recent (e.g. hair and nails) markers of exposure to fluoride to obtain the most reliable biomarker for monitoring fluoride exposure, considering some factors that can influence fluoride uptake and accumulation in these tissues and fluids. The genetics aspect was considered but the results were inconclusive. They are dealt with separately in Appendix 23. Also, for a biomarker of fluoride to be applicable in a large population, it should be easily collectible without objections from the subject. In this regard, this research will also assess the people's preference for the collection of these biomarkers among different age groups and ethnicities.

CHAPTER 3: AIMS AND OBJECTIVES

3.1 MAIN AIM

The overall aim of the study was to find the most acceptable, feasible and reliable biomarkers of exposure to fluoride among contemporary and recent biological markers. The overall aim was investigated by undertaking two separate studies.

3.2 STUDY I

3.2.1 Aim and Subsidiary aim

The aim was to investigate people's perception of collecting biological markers for assessing fluoride exposure among different age groups in the UK. The subsidiary aim was to measure the reliability of the biological markers.

3.2.2 Objectives

- Investigate the most acceptable biomarker of fluoride exposure by age group (4-5 and ≥20 years) using questionnaires.
- Evaluate the feasibility of collecting biological markers of fluoride exposure by age group (4-5 and ≥20 years).
- Evaluate the willingness of participants in providing any of the biological markers among the age groups (4-5 and ≥20 years).

3.3 STUDY II

3.3.1 Aim and Subsidiary aim

The aim of the study was to find the most reliable biological marker (s) of exposure to fluoride among contemporary and recent biomarkers. The subsidiary aim was to investigate people's perception of collecting biomarkers of fluoride exposure by age group (4-5 and ≥20 years) in Nigeria using questionnaires.

3.3.2 Objectives

- Measure total fluoride intake from food and drinks in various age groups (4-5 and ≥20 years) by Food Frequency Questionnaires (FFQ).
- Measure fluoride in 24-hour urine, whole saliva, plasma, hair, nails (fingernail and toenail) and blood in children and adults.

- Investigate correlations between total fluoride intake and i) urinary fluoride excretion (mg/kg bw/d), ii) fluoride concentration in hair (µg/g), iii) fluoride concentration in nails (fingernail and toenail) (µg/g), iv) fluoride concentration in saliva (µg/l), v) fluoride concentration in blood (µg/l).

- Investigate correlation of daily urinary fluoride excretion (mg/kg bw/d) and i) fluoride concentration in saliva (µmol/l), ii) fluoride concentration in blood (µmol/l), iii) fluoride concentration in hair (µg/g) and iv) fluoride concentration in nails (fingernail and toenail) (µg/g).

- Investigate the most acceptable biomarker of fluoride exposure by age group (4-5 and ≥20 years) in Nigeria using questionnaires.

The overall subsidiary aim was to look for differences in the acceptability of biomarker collection between the UK and Nigeria. This was investigated in a separate study:

3.4 STUDY III

The aim of the study was to investigate the relationship between people's perception of biomarker collection in both Studies I and II.

CHAPTER FOUR: OVERALL METHODOLOGY

4.1 INTRODUCTION

This chapter presents the methods used for both study I and study II conducted in the United Kingdom and Nigeria respectively as well as the project phases involved. The chapter is divided into three phases, the first part contains the study design for both studies itemised separately under the following headings: ethical consideration, study location, study population, sample size and study plan. The second part of the chapter provides information on the general analytical methodology including sample preparation, sample transportation, fluoride analysis of samples, data handling, and data analysis. The chapter concludes with the validation of the analytical method used for the determination of fluoride in samples and completeness of 24-h urine samples.

4.2 STUDY DESIGN

This study was conducted in the United Kingdom (UK) and Nigeria. To address the overall aim of the project as described in Chapter 3, both qualitative and quantitative methods were used. Figures 4.1 and 4.2 present overall plans for study I and study II, respectively. In study I, the qualitative part preceded the quantitative part, while for study II, the two parts were conducted simultaneously. The qualitative survey involved the administration of a questionnaire completed either online or as hard copy. The quantitative study involved collection of some selected biological markers of exposure to fluoride from volunteers who were willing to take part.

4.3 STUDY I (UNITED KINGDOM)

4.3.1 Ethical consideration and approval

Ethical approval: The study was approved by Health and Social Care Ethics Committee, Teesside University (Study number 065/15) and the Faculty of Medical Science Ethics Committee, Newcastle University (Study number 00878/2015).

Other approvals: Other relevant approvals were also obtained prior to the commencement of the study. For the qualitative study, approval was obtained from the head teachers of some primary schools in Middlesbrough to circulate questionnaires. However, the researcher was only allowed to pass the questionnaires to the head teachers who then passed them on to parents of children aged 4-5 through their children. Permission to circulate the

online questionnaire was granted by the Dean of each department within Teesside University and Newcastle University prior to the commencement of study. However, for the quantitative study, all participants completed the consent form after careful consideration of the study information documents and the researcher has answered all questions they had. Only participant who completed the consent form took part in the study. Details can be found in chapter 5.

4.3.2 Study Location

The study was conducted in the North-East of England, which has both fluoridated and non-fluoridated areas. More details are presented in section 5.3.3.

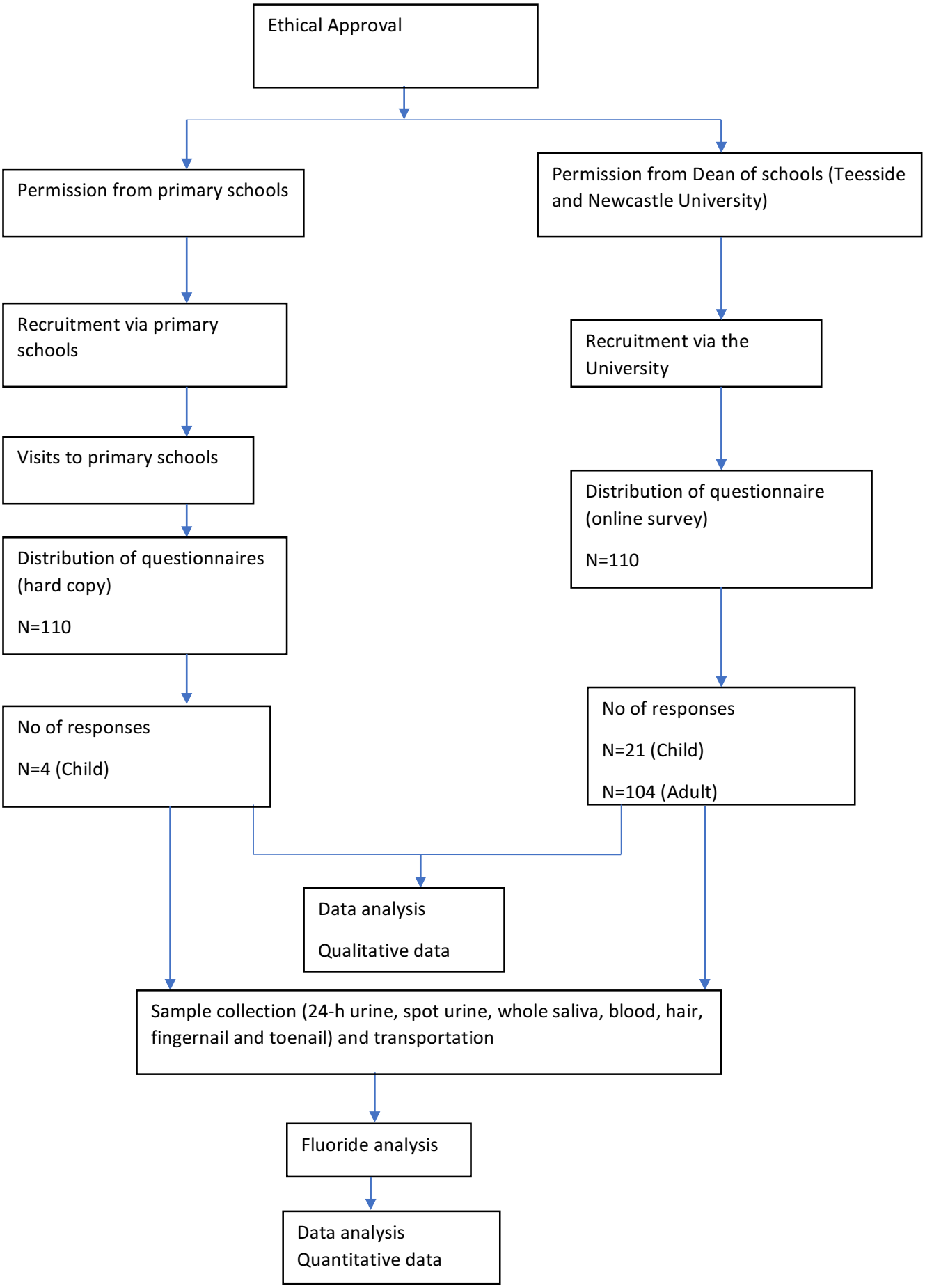

Figure 4.1 Study I flow chart

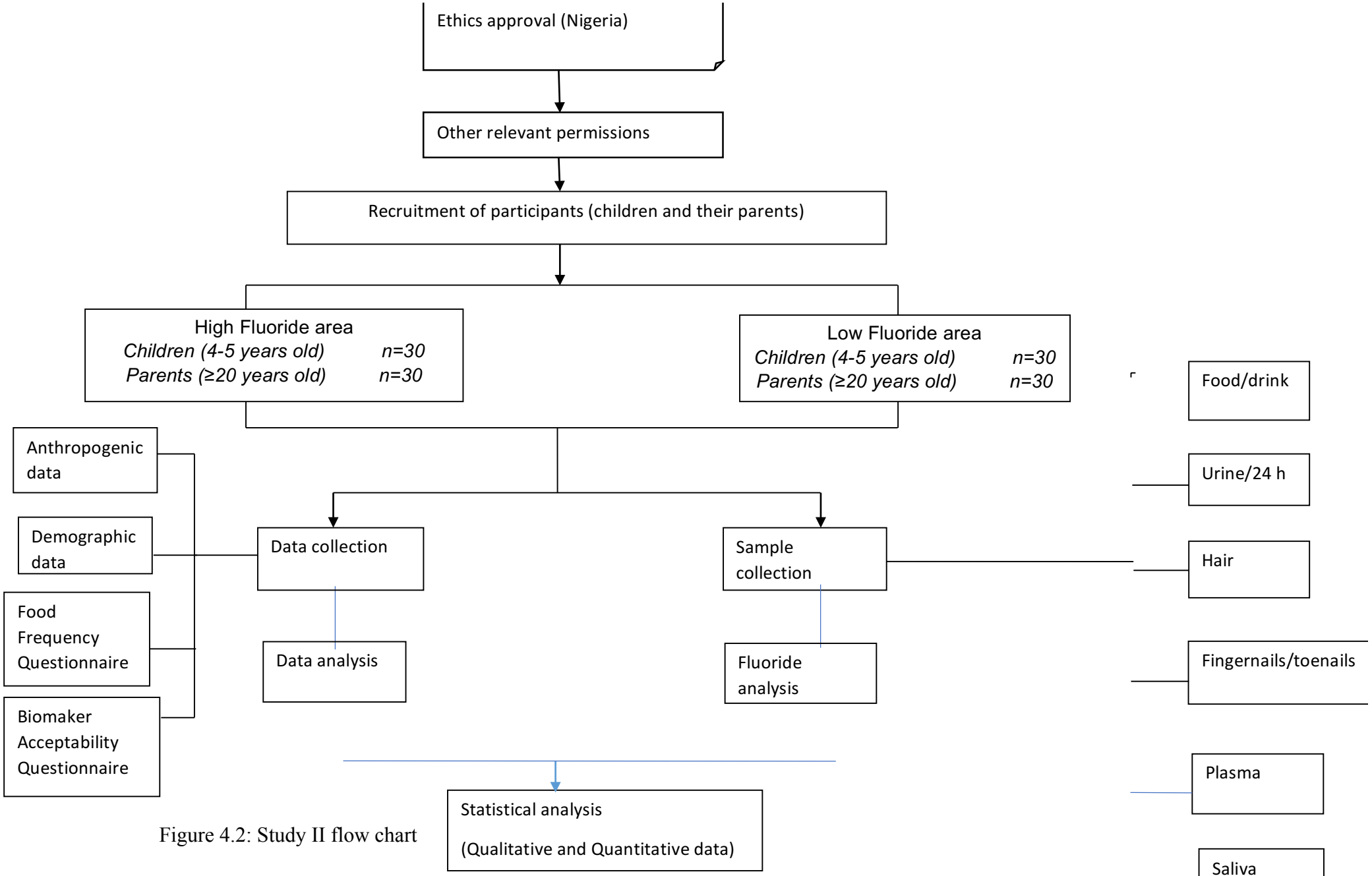

Figure 4.2: Study II flow chart

4.3.3 Study population

The population for this study was children aged 4-5 years old and adult ≥ 20 years old living in Middlesbrough and Newcastle. When estimating fluoride body burden, it is not sufficient to know the amount of fluoride that is ingested but more importantly the systemic bioavailability of fluoride during the critical period of tooth development. Enamel fluorosis on the aesthetically important anterior teeth is caused by excessive fluoride ingestion in the first five years with the most critical period being the first 3 years (O'Mullane *et al*., 2016). It is very difficult to evaluate total fluoride excretion in small children therefore, in the present study, children aged 4-5 years were selected due to being able to control their bladder and not wet themselves at night. This group was targeted mainly because of considerable difficulties in evaluating total daily fluoride intake as well as collecting 24-hours urinary fluoride excretion from infants and young children. The severity of the condition depends on the dose (how much), duration (how long) and timing (when consumed) of fluoride intake. The study population was selected from children in Middlesbrough schools aged between 4-5 years and their parents as well as staff of Teesside and Newcastle universities and their children aged 4-5 years. However, adults were also selected as data will be useful for future monitoring of dental caries and some disease condition that might be associated to fluoride exposure in a population including hypothyroidism, cancer, etc. Information on fluoride retention in human tissues and fluids among children and adults is necessary to obtain reasonably good estimations of community-based total daily fluoride intake and daily fluoride retention.

4.3.4 Sample size

In total, 220 participants from the two different age groups were targeted: 110 children aged 4-5 years old and 110 adults aged ≥20 years old.

4.3.4.1 Qualitative study

The sample size was estimated based on the differences in mean value of acceptability resulting from the two independent age groups and on the level of acceptability for the collection of the biomarkers. An assumption of a moderate effect size relationship between acceptability of collecting biomarkers and age group was used to calculate the sample size due to no previous research on the acceptability of biomarkers. A statistical significance level $\alpha = 0.05$ was also used, suggesting 5% risk that the null hypothesis will be rejected even when it is true, and a power of 85% was assumed. After feeding these values into the

G-power software 3.1 (Manova: repeated measure within-between interactions): a tool to compute statistical power analyses for many different *t* tests, *F* tests, $\chi 2$ tests, *z* tests and some exact tests (introduced by Faul *et al*. 2007): a total sample size of 220 was obtained. This sample size of 220 was used for the study which was shared among the two age groups (adults and children).

4.3.4.2 Quantitative study

The sample size depended on the number of people who were willing to provide samples, which was obtained from the response form. They were sub-set of the larger group that took part in the questionnaire survey and completed the response form. The power of the sample was calculated at the end of the study.

4.3.5 Development of questionnaire (Biomarker Acceptability Questionnaire)

4.3.5.1 Hard copy questionnaire

The Biomarker Acceptability Questionnaire (BAQ) used for this study was developed by the researcher and contains two parts: socio-demographic questions and perception questions. The perception questionnaire was bespoke, but the socio-demographics were collected using standardised categories/criteria. The questionnaire (Appendix 16 and 17) was developed by combining two standard scales including a graphical rating scale and the Likert scale for measuring acceptability and feasibility in children and adults, with addition of a small number of questions relating to biographical details. These details included information on: the year of birth, ethnicity, religion, gender, postcode sector of the residence of the participant (the outward postcode or outcode). The questions were extracted from standard questionnaires that had already been validated from Scottish and English surveys (Office of National Statistics, 2011; Office of National Statistics, 2015). The questions on acceptability and feasibility of the biological markers were designed based on some factors that influence human behaviour (Abdullah *et al*., 2016) including: perceived ease of use, perceived usefulness, attitude towards using, behavioural intentional use and actual use.

The questionnaire was pre-tested informally among a small sample of colleagues in the School of Health and Social Care, Teesside University to establish the feasibility, applicability and acceptability of the questionnaire among the age group and appropriate amendments were made based on the feed-back obtained. For practicality of study, since parents of children aged 4-5 years old were targeted, the follow-up would be possible if

parents were handed paper questionnaires packaged in the bag of their children from the school. The researcher could easily contact the teacher to check if they had been returned. Also, the fact that the study also targetted participants from highly deprived areas, the researcher assumed some parents might not have access to internet, computers or smartphones which would have made it possible to complete an online questionnaire.

4.3.5.2 Online copy questionnaire

After the required amendments were completed on the hard copy following the pre-test, permission was obtained from the Graduate Research School (GRS), Teesside University, UK to administer the questionnaire on the platform of the Bristol Online Survey. The GRS set up a profile on behalf of the researcher and a username and a password was generated for the researcher. The password could be changed by the researcher after activation of the profile. The researcher then uploaded each survey question from the developed hard copy questionnaire onto the Bristol Online Survey platform following the recommendations provided on the platform. Once all questions were uploaded, the researcher launched the survey to be completed within a certain period. The link to the survey was copied into all emails sent to staff of Teesside and Newcastle Universities. This online method was selected by the researcher for university participants, as it was believed that it would enhance the response rate and because it would be difficult to ensure a paper questionnaire was received by the staff, and subsequent follow-up might be difficult. Also, staff had the choice to complete a hard copy of questionnaire if they wished.

4.3.6 Study plan

This study consisted of two phases, namely: quantitative and qualitative. Qualitative data were obtained through questionnaires given to the participants or completed online (depending on the participants as discussed above) and the quantitative data were obtained when participants, who were willing to provide any of the biomarkers, provided the samples for estimation of exposure to fluoride. In this UK study, participants for the quantitative phase were a sub-sample from the qualitative phase, who chose among the biomarker(s) those they were willing to provide.

4.3.6.1 Part one: Qualitative study

Recruitment of children: The researcher visited the schools to meet with the head teachers of the selected primary schools where he handed out the study packs, which were distributed by the head teachers to the children aged 4-5 years to pass on to their parents. A date was arranged for the collection of the completed questionnaires.

An email with a link to the survey was also sent to the staff of Teesside and Newcastle Universities for them to complete on behalf of their 4-5 years old children.

Recruitment of adults: An email was also sent to the staff of Teesside and Newcastle Universities containing an invitation to participate in the study, a link to an online survey and soft copy attachments of the questionnaire and an opportunity for future research details/response form. Those who were willing to participate completed the survey online, others completed the soft copy of the questionnaire and returned it to the researcher via email.

Parents of children aged 4-5 years old selected from Middlesbrough schools were also invited to participate in the study. A copy of the questionnaire was attached to the study packs passed to the parent by the head teachers for the parents to complete for themselves.

4.3.6.2 Part two: Quantitative study

Those who participated in part one were invited to take part in the second phase (part two). The inclusion criteria for this part were: aged 4-5 and ≥20 years old of either gender; with no dietary restriction; no chronic or metabolic disease and urinary infection; no oral disease (no tooth or gum pain) and no professional dental treatment such as the use of fluoride varnish for at least three months prior to the start of the study; parental consent for children. Volunteers who met the inclusion criteria were invited for a meeting in a convenient place where the details of the study were discussed and they asked the researcher for clarification of any information they might not understand. Volunteers were given two to three days to think about participating. Once they agreed, they contacted the researcher by phone and a visit was arranged at a convenient place. At this meeting, participants were given the medical history form to complete and the details were discussed upon completion. Those who met the medical requirement were given the consent form to complete. Participants were then invited for two more visits:

Visit one: Participants visited the School of Health and Social Care, Constantine Building, Teesside University where they completed the demographic and Fluoride exposure data form including: name, home address, month and year of birth, gender, tooth brushing habits information. The height and weight of participants were measured and recorded on the anthropometric data collection sheet during this visit. Height and weight were measured without shoes using a Telescopic measuring rod for seca column scales (Seca Mod. 220) to the nearest 0.5 cm and 0.1 kg respectively. Participants were then provided with the biomarker collection kits to use at home or anywhere convenient. Instruction was also provided for collection of sample(s) (including any of blood, urine, saliva, hair and nails) and for the completion of the questionnaire; participants were asked to return them on the second visit. Participants only gave samples they were willing to provide which was indicated on the acceptance form. All samples were collected by the participants (or by their parents for 4-5 years old children) except blood samples which were collected by a trained nurse in the School of Health and Social Care, Teesside University during the first visit for those participants who were willing to provide samples.

Visit two: This visit was arranged at a place convenient for the participant when all biomarker samples collected at home were collected by the researcher including: saliva, 24-h urine sample, spot sample, hair, fingernail and toenail samples. The Food Frequency Questionnaire (FFQ) was also completed during this visit. The researcher ensured all food and drink items were entered correctly into the questionnaire. The weight of food and drink consumed by the participant was estimated using a food portion atlas (Nelson *et al*., 1997). [The food atlas shows the type of food and drink items and household measures as life size pictures.] The samples of biological markers were transported in a cool box to the fluoride laboratory in the School of Health and Social Care, Constantine Building, Teesside University by the researcher, where they were prepared and stored until analysis. A water sample (about 10 ml) for fluoride analysis was collected from the participants' primary source of drinking water during this visit.

4.4 STUDY II- NIGERIA

4.4.1 Ethical consideration and approvals

Ethical approval: The study was approved by the School of Health and Social Care Ethics Committee, Teesside University, UK as well as the University of Jos Teaching Hospital, Plateau State, Nigeria.

Other approvals: Approval was also obtained from the Ministry of Health, Plateau State and the Hospital Board Management, Plateau State. As part of these approvals, the researcher was given permission to recruit support staff, including a phlebotomist and laboratory assistant, and to use the hospital facilities, including work station and analytical equipment. Verbal permission was also given by the Local Government Authorities as well as the district heads and relevant community heads of the selected villages prior to the commencement of the study.

4.4.2 Study location

The study was conducted in North-Central Nigeria which has naturally fluoridated and non-fluoridated areas. Bokkos Local Government Area (LGA) with a low fluoride concentration in the drinking water, less than recommended by the World Health Organisation (WHO), and Langtang LGA with fluoride in the drinking water higher than that recommended by WHO were selected. The fluoride concentrations of drinking water in Bokkos and Langtang LGAs were 0.028 and 3.36 mg/l respectively, from the preliminary study results (See Chapter 6). The low and high fluoride areas were selected to determine the sensitivity of the selected biomarkers when the concentration of fluoride is low and to provide information on the levels of fluoride in biomarkers when exposure is high. This might be useful in future monitoring particularly for endemic areas to determine children at risk.

4.4.3 Study population

Healthy volunteers consisting of children aged 4-5 years, and their parents aged ≥20 years were recruited from selected communities in Bokkos and Langtang Local Government Areas (LGA) of Plateau State, Nigeria with low and high fluoride in drinking water respectively. These LGAs were selected following a preliminary study conducted in the area where samples of drinking water were collected randomly by the researcher from different sources in the study area and analysed for fluoride in the UK. This preliminary work is fully discussed in chapter 6.

Other inclusion criteria were: having no history of metabolic disease (e.g. diabetes) or acid-base disturbance; not receiving a therapeutic/restricted diet or recent professional fluoride application; not suffering from gastrointestinal disorder, bone, renal problems, or having urinary tract infections and not being on certain medications. Partcipants who did not meet the above criteria were exempted from the study.

4.4.4 Sample size

A total of 120 participants were recruited distributed among the 4 target groups (30 children 4-5 years) with their parents (30) in the high fluoride area and 30 children (4-5 years) with their parents (30) in the low fluoride area). To estimate the sample size, a two-tailed test was selected for the study to examine if the means of each biological marker (urine, blood, nails, hair and saliva) are different from each other in the high fluoride area and low fluoride level. The means were compared using ANCOVA on the G-Power software package. An effect size of 0.851 was used, based on previous studies which showed a highly significant correlation between increasing water fluoride concentration and the level of fluoride in the biomarkers (Appendix 24). A statistical significance level α of 0.05 was assumed, which suggested that a 5% risk of accepting the null hypothesis when in fact it is true required a sample size of 80 which will be shared among the 4 groups consisting of child and parent in high and low fluoride area. The power of the study used in estimating the sample size was assumed to be 90%. A drop-out rate of 30% to give a total sample size of 102 was assumed. However, the plan is to recruit 120 participants in total representing 30 participants for each group. A total sample of 60 children with their parents of both genders were recruited. The informed consent and assent forms were attached to the study pack parents received during the introductory session. All participants were given time to consider taking part before they were invited to attend an introductory session in the community centre or village clinic where other questions were answered by the researcher and an abbreviated medical history form was completed. The researcher then collects the completed consent form from parents who meet the eligibility criteria and are happy to take part in the study as well as their child. The consent form was signed after all the questions have been answered by the researcher prior to the commencement of the study. Volunteer whose literacy level is low, on the day of visit to the village, the researcher explained verbally the study information and informed consent. The inform consent was kept in a secure cabinet in Federal College of Forestry Jos, Nigeria by the researcher. Only volunteers who complete the consent form took part in the study. Participants who do not meet the inclusion criteria were excluded from the study.

4.4.5 Study plan

4.4.5.1 Recruitment of support staff

There was a need to recruit a phlebotomist in the field since the researcher was not trained to collect blood samples. Also, field interpreters were required for the practicality of the

study. The level of education of most of the participants living in the study location was low and there was also a language barrier between the researcher and the participants. Written instructions were provided for the study, however, due to their level of education participants might not have been able to understand why a piece of information was necessary and how to record such information accurately. In this regard, the researcher recruited interpreters who were then trained in the research procedure and how to collect the data. These interpreters guided the participants' one on one on how to collect the samples and why they must adhere to the procedures. In this way, mistakes were avoided as the participants could ask questions surrounding their livelihood and how it could affect the collection of the samples. The participants could then be advised appropriately by the interpreters, which would not have been possible with written instructions. In this study, where most of the data were collected through the questionnaire including FFQ, BAQ, Oral hygiene questionnaire, there might have been a problem compiling the data if they were not completed accurately. Similarly, there was a clear guideline for sample collection which, if not performed as instructed, would have affected the validity of the data.

Adverts were placed in the general hospitals of the selected LGAs for support staff including phlebotomists and interpreters, and interviews were conducted (details in chapter six). The successful candidates were trained in the procedures involved in the research and each role was identified. All questions were answered by the researcher at the end of the training.

4.4.5.2 Recruitment of participants

Consultations were made with parents of 4-5 years old children in a meeting organised by the village heads of each community where the researcher explained the details of the research and answered all questions parents had. Parents were given time to consider taking part in the study until an arranged date when those who were interested signed the consent form on behalf of themselves and their child. The researcher ensured that each item of information on the consent form was carefully considered before the parent consented. The details are discussed in Chapter 6. Participants then completed the Medical History form which was used to assess their health. Any participant that was not healthy following these assessments was dropped and did not take part in the study. All participants also consented to the study prior to its commencement.

4.4.5.3 Data collection

Two visits were organised with the participants:

Visit one: The participants, including parents as well as their children, were invited to attend a general community centre (village centre or medical unit of the village) when general information (including date of birth and age), tooth brushing habits (e.g. type of regularly used toothpaste, frequency of brushing per day) were collected. The initial Biomarker Acceptability Questionnaire (BAQ) was also completed on the first visit. The weight and height of participants was measured and recorded on the anthropometric data collection sheet during the first visit. Height was measured using a portable stadiometer (DE56618903; ADE, Germany) vertically without shoes to the nearest 0.5 cm. Weight was measured using a portable digital scale (seca 803, Seca, Germany) without shoes to the nearest 0.1 kg. The equipment used were properly calibrated, however, the same weight and height scales were used throughout the study to avoid any possible measurement errors. The parents were then given two sample-collection packs (consisting of: zip-locked plastic bags for collection of fingernails and hair; bottles and containers for collection of water; a funnel, plastic bottles and several disposable cups to collect urine samples; and written instructions on how to collect the samples. (This was explained verbally and demonstrated on visits to those with a low literacy level).

Visit two: A second visit was then arranged with all the participants at the village centre or community medical centre. Participants were told not to eat or drink anytime 2 hours prior to the visit. During the visit, 24-hour urine, samples of hair, nails (fingernails and toe nails), and saliva were collected. Parents were interviewed by the researcher and trained staff to complete the Food Frequency Questionnaire (FFQ) due to their literacy level. The collected samples, including: food, drinks, fingernails, hair, saliva and 24-hour urine, were transported by the researcher to the storage facility in Jos, Plateau state where they were stored until analysis. Participants also provided fasting blood samples (~ 10 ml) collected by a qualified nurse. On this visit, after participants had provided all the samples, they completed a second Biomarker Acceptability Questionnaire (BAQ).

4.5 GENERAL ANALYTICAL METHODOLOGY

4.5.1 Sample collection and preparation

In the present study, specific biomarkers were considered by the researcher for their ability to reflect the body burden of fluoride: including blood, saliva, teeth, bone and urine (Buzalaf et al., 2011). However, teeth and bone were not selected due to the difficulty in obtaining such samples, which might have required a biopsy which is also an invasive procedure. Also,

nails (fingernail and toenail) and hair were selected due to their ability to reflect past blood fluoride concentration and the body burden of fluoride (Buzalaf et al., 2011).

4.5.1.1 Sample collection

24-h urine collection: Participants were given written instruction on how to collect 24-h urine samples, two labelled disposable plastic bottles (1000 and 500 ml) with screw caps, a disposable funnel, several disposable cups and a jug on their first visit. The reason for the 24-h collection was explained and why it was necessary to collect all voided urine over a 24-h period was stressed. Participants were asked to discard the first voided urine in the morning of collection day but to record the time, which was marked as the beginning of the 24-h urine collection. All other urine passed was collected and the first of the following day was marked as the end of the 24-h urine collection. Urine samples were picked up by the researcher on the second visit.

Spot urine collection: Written instructions were given to participants prior to the collection. The participants urinated at several times of the day and collected a small sample of it in a container each time. The container was sealed and given to the researcher on the second visit.

Whole saliva collection: The collection of whole unstimulated saliva was performed by the participants with written instructions. Participants were asked not to eat or drink anything (other than still water) or use toothpaste/mouth rinses within 4 hours of saliva sample collection. Unstimulated whole saliva was expectorated for a period of 3 minutes into a funnel placed in a graduated plastic tube. Saliva samples were then collected by the researcher on the day of visit.

Blood collection: The collection of blood was done at the School of Health and Social Care, Teesside University on an arranged date with the participant. Participants were asked to fast overnight prior to blood sample collection. Blood samples (approximately 5 ml but in Nigeria an extra 2 ml was collected to provide sample for DNA analysis for participants who consented) were collected from participants with the assistance of a qualified nurse, following standard medical practice for collection of blood samples from the arm. The nurse obtained the identity of the participant, confirmed the amount of blood that would be obtained and explained the procedure. Then the nurse exposed the site of collection in the arm and applied a hand band to the upper arm and identified a vein in the lower arm where

a syringe needle was inserted through the skin and into the vein. The needle was held firmly in the vein with one hand and an appropriate blood tube was inserted into the syringe barrel to collect the blood sample. After obtaining the blood, the nurse removed the specimen tube gently whilst holding the needle firmly in place. The nurse then removed the tourniquet and placed a dry cotton wool ball over the needle when withdrawing the needle. The needle and the holder (still connected) was disposed of in the sharp bin. The specimen was completely and accurately labelled (date, ID code) and stored in the appropriate bag kept in a waterproof box used in transporting to where analysis of fluoride was conducted. The nurse ensured that the wound had stopped bleeding, then applied a plaster on the puncture site.

Hair collection: Following written instructions, participants willing to provide hair samples collected a few milligrams of hair about 5 cm in length with the help of parents/partners/ etc. Hair was cut as close to the scalp as possible with clean scissors (care was taken not to nick the skin) from the back of the head and sealed in an envelope provided by the researcher. The sample was given to the researcher on the day of visit.

Nail collection (finger and toenail): Following written instructions on how to clip fingernails and toenails, they were clipped by participants willing to provide nail samples. Fingernail clippings were stored separately from the toenails in zip lock bags that were provided by the researcher and stored at room temperature before transport to the laboratory by the researcher.

Food and drink samples:

There were several dietary assessment methods available, as previously identified in Chapter 2. However, some of these methods would not have been practical for the researcher to use at the study location. The market basket would have been difficult for the researcher to use in the present study as it would have been time-consuming considering the limited time available to the research to spend in the field for data collection. There were four study locations to be investigated in Nigeria, and this meant that the researcher would spend at least 14 days in each for food data collection. Also, observing food consumption is time-consuming and would have required much effort from the researcher having to observe the dietary habits of the participants. This was impossible for the field studies due to the distance of the researcher's base from the paticipants' location. The duplicate method is also expensive and could not be implemented in the study. The participants might have been forced to save some money provided for duplicating their food and therefore influenced their

usual dietary habit by eating a cheaper food during the period of data collection. For 24-hour dietary recall, considering that the present study was investigating long-term biomarkers, this method may not have represented the long-term dietary habits of the participants. Diet history would have been an appropriate method, but not all participants in the present study could read and write and therefore it would have been difficult to record their dietary information in the pocket-sized diary provided.

Therefore, the researcher selected a food frequency questionnaire due to its low cost, ease of use and efficiency to assess the usual diets of population groups. Some limitations are associated with the use of FFQs, including: participants' error filling out the questionnaire and no way to find out how foods are prepared, over-reporting and under-reporting by the participants. To mitigate some of these limitations, the researcher ensured the recruited interpreters completed the questionnaires while the participants were interviewed due to the low educational level of most participants. Also, to ensure all ingredients used for the preparation of food were considered, the researcher advised some of the participants to prepare all food identified in the FFQ their usual way which was analysed for fluoride. There might still have been a chance or over-reporting or under-reporting but this was the most practical method for collection of dietary information in the study location.

The samples collected included:

a. Home-made food and drinks: Each home-made food from the FFQ was provided by selected participants and homogenised using a hand blender. The sample was then divided into a couple of small zip-lock plastic bags, labelled with the food code and fluoride area the sample was collected from and stored at -20^{o}C prior to analysis.

b. Preparation of readymade food/drinks items: These were identified in the FFQ, purchased and prepared for fluoride analysis in-line with a modified study protocol used in Newcastle University (Zohoori *et al*., 2012; Martinez-Mier *et al*., 2011). Samples of each food and drink were purchased from different stores in Nigeria. Equal weights/volumes (100 g or 100 ml) of each food or drink was measured and thoroughly mixed to provide a homogenised sample for subsequent fluoride analysis. The samples were then divided into two labelled zip-lock plastic bags and stored at -20^{o}C for fluoride analysis.

4.5.1.2 Samples collected in the United Kingdom: During the second visit at a convenient place, the researcher collected the samples from the participants and transported them to the

fluoride laboratory (C 1.11) in the School of Health and Social Care, Constantine Building, Teesside University. There was no restriction on the type of biological maker the participants were happy to provide as it was entirely up to them. Samples of urine, hair, nails (fingernails and toenails) and saliva were then prepared according to the procedures written below. Food and drink samples were not collected from participants in the United Kingdom, only water samples.

4.5.1.3 Samples collected in Nigeria: During the second visit at the community centre or the village clinic, the researcher collected all the samples provided by the participants and transported them in a cool box to the Chemistry laboratory, Federal College of Forestry, Jos, Nigeria. The samples including food and drinks, blood, urine, saliva, hair and nails (fingernails and toenails) were then prepared according to the procedures.

4.5.1.4 Sample preparation

Urine: All urine samples collected over the 24-hour period were pooled for each participant and the total volume was measured and recorded in a urine record form. Two aliquots of 7 ml were then taken, one for analysis of fluoride and the other kept as back up. The containers were appropriately labelled with child ID, type of sample and date of collection. The samples were then stored at -20^{o}C until analysis.

Saliva: Each collected saliva sample was centrifuged at 1000 rpm for 2 minutes to allow separation of any food debris (Martinez-Mier *et al*., 2011). A 5 ml pipette was then used to collect the supernatant into a 7 ml bijoux tubes coded with the child's ID. The samples were then stored at -20^{o}C until analysis.

Plasma preparation: The collected blood samples were immediately taken to the laboratory in cool boxes. Each blood sample was centrifuged for ten minutes at 1500 rpm by centrifuge (Harrier 18/80). The plasma samples for each participant were then collected gradually using a 1 ml pipette into an individual 7ml bijoux tube with participant ID. The samples were then stored in the freezer at -20^{o}C for later analysis. For blood samples collected in Nigeria, they were divided into two prior to plasma preparation. A 2ml sample of whole blood was used for extraction of DNA (see details in Chapter 6) and plasma was separated from the remaining 8 ml as described above.

Nail (Fingernails and toenails): The fingernail clippings were prepared separately from the toenail clippings. However, the same procedure was used. Each nail sample was first

weighed and transferred into a 5 ml bijoux tube coded with the participant's ID. Five ml of double de-ionized water (DDiH2O) was added into the bijoux tube. The container was placed in a sonicator for 15 minutes, then the dirty water was disposed of. The clean nail samples were then transferred into a clean crucible coded with the participant ID and heated at 55^{o}C for 3 hours in an oven until a dried sample was obtained. The new weight was then measured using an analytical balance and recorded in the nail record sheet. Each sample was then transferred into a ceramic mortar and a sufficient quantity of liquid nitrogen was added to immediately grind the nail samples into powder. The final weight was then measured and recorded. The samples were then transferred into bijoux tubes coded with participants' ID and stored at room temperature until analysis.

Hair: Each hair sample was weighed and the weight recorded. The samples were transferred back into their respective zip lock bags and stored at room temperature until analysis.

4.5.2 Sample transportation

4.5.2.1 Samples collected in the UK

Samples in the UK were collected by the researcher within Teesside University and transported in a cool box to the School of Health and Social Care, Constantine Building, Teesside University (C 1.11) except hair and nail samples stored at room temperature. Blood samples were collected in the physiological laboratory in the School of Health and Social Care, Constantine Building, Teesside University (C 1.08) and transported to C 1.11 as described above.

4.5.2.2 Samples collected in Nigeria:

Transportation in Nigeria: Sample transportation in Nigeria was planned prior to the commencement of the study due to the distance between study location and storage facility as well as the laboratory used for analysis to protect the integrity of the samples. Collected blood and tissue samples were sealed inside a water proof box and labelled properly (name of samples, date of collection and identity code). The researcher ensured appropriate packing of the blood samples to absorb spillage if the vials leaked. All samples collected including urine, saliva, hair, nail (fingernail and toenail), blood was transported in a cool box by the researcher's private car to the storage facility in Jos, Plateau state where they were stored at -20^{o}C after preparation except hair and nail samples that were stored at room temperature and then transported to the Chemistry laboratory, Federal College of Forestry, Jos Nigeria

for fluoride analysis. The water proof box was kept in the boot during transportation. In the high fluoride area, samples of urine and saliva were prepared and analysed immediately on arrival from the study location in Langtang general hospital while plasma (for fluoride analysis) as well as blood (for DNA analysis), hair and nail samples were transported to the storage facility in Jos as described above.

Transportation of samples to the United Kingdom from Nigeria: All hair and nail samples were stored at room temperature in zip lock bags and labelled appropriately and transported to the UK in the researcher's luggage. Extracted DNA samples were labelled appropriately and kept frozen at -20^{o}C in Plateau State Institute of Virology (PLAVIREC) prior to transportation to the UK. The frozen DNA samples were kept in a water proof box loaded with dry ice and labelled appropriately for transport in the researcher's luggage to the UK. Upon arrival, hair and nail samples were stored at room temperature in the fluoride laboratory, School of Health and Social Care, Teesside University. In the package of frozen DNA samples, no ice was left and the samples were thawed. The DNA samples was then transported to the Oral Biology laboratory, Newcastle University in the researcher's private car and stored at 4^{o}C prior to analysis.

4.5.3 Fluoride analysis

4.5.3.1 Analyses conducted in UK

Fluoride analysis of all samples was conducted by the researcher in the Fluoride laboratory, Constantine building, School of Health and Social Care, Teesside University. Water and urine (24-h and spot urine) samples were analysed directly using a fluoride ion selective electrode (Martinez-Mier *et al*., 2011). Blood, whole saliva, hair and nail (fingernails and toenails) samples were analyzed using a modification of the hexamethyldisiloxane (HMDS) method (Whitford, 1996). The fluoride content of each sample was obtained by comparison of the millivolt reading of the sample to a standard curve prepared from the data of diffused fluoride standard solutions analyzed at the same time. All samples were analyzed in triplicate and waste discarded appropriately. Blood, saliva and urine samples were analyzed within 48 hours of collection. Data obtained were recorded in an Excel spreadsheet.

4.5.3.2 Analyses conducted in Nigeria

Fluoride analysis of samples including water, urine, saliva, plasma was conducted by the researcher in the Chemistry laboratory, Federal College of Forestry, Jos Plateau state. Water,

24-h urine and saliva samples were analysed directly using a fluoride ion selective electrode (Martinez-Mier *et al*., 2011) while blood samples were analyzed using a modification of the Hexamethyldisiloxane (HMDS) method (Whitford, 1996). Hair and nails (fingernails) were analysed for fluoride in the UK. The fluoride content was obtained by comparison of the millivolt reading of the sample to a standard curve prepared from data of diffused fluoride standard solutions analyzed at the same time. Samples were also analyzed in triplicate by the researcher and waste discarded appropriately according to the standard laboratory procedure of the Federal College of Forestry, Jos Plateau state. Data obtained were recorded in an Excel spreadsheet.

4.5.3.3 Analysis of samples

Urine/water: The electrode was calibrated using a series of standards whose concentration were chosen to ensure that they covered the expected range of sample concentration (Venkateswarlu & Vogel, 1996). Each standard was mixed with TISAB III in a proportion of 1:10 (v/v) in a bijoux tube prior to analysis. Frozen samples were defrosted at room temperature and fluoride concentrations of samples were measured in triplicate by a direct method of analysis using a F ion-selective electrode (Model 9409 Thermo Orion, USA) and a pH meter (Model 720) after addition of TISAB III (Martinez-Mier *et al*., 2011).

Saliva: Frozen saliva samples were defrosted at room temperature prior to fluoride analysis. Fluoride concentrations of the samples were measured by the above-mentioned direct method in triplicate, at room temperature using F ion-selective electrode and meter after adding TISAB III. Prior to measuring the sample fluoride concentrations, the electrode was calibrated using a series of standards prepared by adding TISAB III in a proportion of 1:10 (v/v). Concentration of fluoride standards was chosen to ensure that they covered the range of expected sample concentrations (Martinez-Mier *et al*., 2011)

Plasma: Frozen plasma samples were first defrosted at room temperature. The fluoride concentration was measured by F ion-selective electrode after over-night HMDS acid-diffusion in triplicate at room temperature (Taves, 1968; Venkateswarlu & Vogel 1996). The HMDS acid-diffusion was used to release the fluoride ion of the plasma samples as well as the standards between 16-24 hours (Martinez-Mier *et al*., 2011).

Distilled water (1 ml) was placed in a non-wettable diffusion dish and the plasma was added. Known quantities of fluoride standards (e.g. 0.01, 0.1, 1, 10, 100 mg/l in triplicate) were

added to the distilled-deionised water in other dishes. The alkaline trapping solution (50 µL of 0.75N NaOH) was placed in five drops on the inside of the dish lid. Parafilm was then used to seal the lid to the bottom of the dish. Finally, 3.0N H_2SO4 saturated with HMDS (1 ml) is injected through a hole previously burn through the lid near its periphery with a soldering iron and the hole is immediately covered with Parafilm. The diffusion of fluoride from the sample and the standard into the NaOH trap begins at the time. The diffusion process was then allowed to continue for a period of 16-24 hours. The lid was then removed and 20 µL of 0.10N acetic acid was added and combined with the NaOH into a single drop to form a buffered sodium acetate/acetic acid solution with a pH of approximate 5. The solution was drawn into the tip of a Finn pipette and the final volume of solution adjusted to 75 µL with distilled deionised water. The electrode was then placed in contact with the solution with gentle mixing movement of the dish or electrode every 15-20 seconds until a stable mV reading was obtained (Whitford, 2005). The relationship between the mV reading and the concentration (actual activity) of fluoride in the solution was tested in a linear regression curve, using Excel software, where a calculation program transformed the value of mV provided by the electrode into µg F/ml for each plasma sample.

Nail (fingernail and toenail) and hair: The fluoride ion was released from nail and hair samples by overnight diffusion with Hexamethyldisiloxane (HMDS) as described in Table 4.4. Where a sample was ≤10 mg, it was analyzed without replicate, however, when the weight was >15 mg and ≤20 mg, duplicate samples were analyzed and for samples >25 mg and ≤30 mg, they were analysed in triplicate. The left-over samples from nail and hair was used for the recovery and reanalysis meant to validate and test the reliability of the analytical procedure.

The HMDS diffusion process of analysis of fluoride above was also used for analysis of nail and hair, however, 3 ml HMDS and 3 ml distilled water was added to the sample. 50µL of 0.05N NaOH was used for the alkaline trapping and 20 µL of 0.20N acetic acid was combined to produce a buffered sodium acetate/acetic acid solution with a pH of approximately 5 (Table 4.1).

Table 4.1: Summary of analysis: Modification of Taves, 1968; modified by Martinez-Mier *et al*., 2011.

Weight of sample	Volume of DDH_2O	Concentration of NAOH added (50 µl)	Volume of HMDS saturated with H_2SO_4 (3.0 N)	Concentration of acetic acid added (25 µl)	Total Volume for ISE
10 mg sample (Nail and hair)	3 ml	0.05 N	3 ml	0.20 N	75 µl
1 ml plasma	1 ml	0.075 N	1 ml	0.10 N	75 µl
1 ml toothpaste containing SMFP	1 ml	0.05 N	1ml	0.020 N	75 µl
1 ml food and milk based drink	1 ml	0.05 N	1ml	0.020 N	75 µl

Note: Samples were analyzed in triplicate (for nail and hair 10 mg each was used for analysis, no replicate was done for samples ≤10 mg).

Toothpaste: Common brands containing different forms of fluoride were chosen from those sold in the Nigerian market (and depended on what was recorded by the subjects on the toothpaste record sheet). Before analysis 2 cm of each toothpaste was discarded, then a labelled microcentrifuge tube was weighed, the weight recorded (M1) and then 1 g of toothpaste was placed into it (weighed and recorded M2). The sample was made up with 0.9 mL distilled-deionised water and weighed again and recorded (M3). The microcentrifuge tube was then whirled with three acid-washed glass balls added to the tube and centrifuged at 10,000 rpm for 20 min. The supernatant (0.1 ml) was then placed into a 20 ml polystyrene tube and diluted to 10 ml with 9.9 ml of distilled-deionised water. The diluted supernatant was then used for fluoride analysis depending on the form of fluoride in the purchased toothpaste. Toothpaste containing fluoride as sodium fluoride was analysed in triplicate by the direct method after 1:10 (v/v) mixture with TISAB III (Martinez-Mier *et al*., 2011). Toothpaste containing fluoride as sodium monofluorophosphate (SMFP) was analysed after overnight diffusion with HMDS (Whitford, 1996).

Food and drink:

a) Non-milk-based drinks and water: fluoride concentrations of these types of samples were measured directly in triplicate using an F ion-selective electrode after adding TISAB III in the proportion of 1:10 (v/v) at room temperature (Martinez-Mier *et al*., 2011)
b) Food samples and milk-based drinks: the HMDS acid-diffusion technique was used to release fluoride ions from the food samples as well as from set of standard solutions for construction of the calibration curve, overnight (Whitford, 1996). The fluoride concentration of the samples was measured in triplicate, at room temperature.

4.6 DATA HANDLING/PROCESSING

4.6.1 Qualitative data: Upon collection of the questionnaires by the researcher, data were compiled in an Excel spreadsheet. The data were analysed using the Statistical Package for Social Sciences version 22 (SPSS). To effectively manage the data obtained from the hard copy of the questionnaire on the software, items on the questionnaire were first assigned codes by giving them variable names. An SPSS version of the online survey was generated from the Bristol Online Survey (BOS) platform used to administer the online survey. Values for missing data were defined and value labels for categorical variables were defined.

4.6.2 Quantitative data: Upon analysis of fluoride in the biomarker samples, data were compiled in an Excel spreadsheet before transferring to SPSS.

Urine data processing: The volume of urine was corrected for 24 h using the formula below and the flow rate calculated by dividing the corrected urine volume (ml) by 24 hours. Urinary fluoride excretion (UFE) was calculated by dividing 24 h urinary fluoride excretion by the 24 h corrected urine volume and fractional urinary fluoride excretion (FUFE) was calculated by dividing UFE by total fluoride intake from all food and drinks as well as toothpaste.

Corrected urine volume (ml/24 h) $= \frac{Total\ urine\ volume\ (ml)}{Duration\ of\ collection\ (min)} * 1440$, where 1440 is the number of minutes in 24 h.

Urine flowrate (ml/hr) $= (24\ \text{h}\ corrected\ urine\ (ml/24\ \text{h})) \div 24.$

Urinary fluoride excretion (µg/day) $= 24\ \text{h}\ urine\ fluoride\ conc.$ (µg/ml) $X\ 24\ \text{h}\ corrected\ vol.(ml/24\ \text{h}).$

UFE (mg/kg bodyweight/day) $= (UFE\ (\mu g/g)\ \div 1000)\ \div$ weight (kg))

ii) Food and drinks data processing:

Total F intake from diet $(\mu g/day) = Total\ F\ intake\ from\ water\ (\mu g/day) +$
Total F intake from all non milk based drinks (μg/day) +
Total F intake from food and milk based drinks (μg/day)

Total fluoride intake $(\mu g/day) = Total\ F\ intake\ from\ diet\ (\mu g/day) +$
$Total\ F\ intake\ from\ toothpaste\ (\mu g/day)$

Total fluoride intake (mg/kg bw/d) $= (Total\ F\ intake\ (\mu g/day) \div 1000)\ \div$
$weight(kg)$

iii) Toothpaste data processing

Amount of fluoride dispensed (μg) = $weight\ of\ toothpaste\ (g) \times$
$fluoride\ concentration\ of in\ used\ toothpaste\ (\mu g/g)$

Amount of toothpaste swallowed (μg/brushing) = $\left(\frac{41}{100}\right) \times$
$Amount\ of\ fluoride\ dispensed\ (\mu g)$

Where 41% is the assumed swallowed during tooth brushing (Omid, 2002)

Total fluoride intake from toothpaste (mg/brushing) = $(Amount\ swallowed\ (\mu g/$ brushing) $\div\ 1000) \times number\ of\ brushing\ per\ day$

Food weight/day = $(No\ of\ days\ consumed \times Weight\ per\ day) \div 7$

Fluoride ingestion from toothpaste (mg/d) was estimated by multiplying the pictorial recorded weight of toothpaste used per brushing (mg) by its fluoride concentration and frequency of use. The obtained value was multiplied by 41%: the mean % of toothpaste ingested per tooth brushing session reported for children (Zohoori and Runn-Gunn, 2000; Zohoori et al., 2012; Ibiyemi et al. 2016). Due to the lack of data for adults and considering the recommendation "spit don't rinse" by a group of experts who formulated consensus statements regarding rinsing behaviour and the prevention of dental caries (Pitts et al. 2012), the same percentage was used for adults.

4.7 DATA ANALYSIS

4.7.1 Qualitative data: Data were managed using SPSS version 22. To effectively manage the data obtained from hard copies of the questionnaire, items on the questionnaire were first assigned codes by giving them variable names. Values for missing data were defined and value labels for categorical variables were defined.

4.7.2 Quantitative data: The following variables were estimated from the FFQs: average daily intakes of i) water ii) Non-milk based drinks and iii) food and milk based drinks. Body mass index was calculated from anthropometric measurements. Total daily dietary fluoride intake, fluoride intake from toothpaste ingestion, total daily fluoride intake from diet and toothpaste, volume of 24h-urine, urinary flow rate, daily urinary fluoride excretion, and fluoride concentration were estimated in nails (fingernails and toenails), hair, plasma and saliva. See details in Table 4.2.

Table 4.2: Table of outcome variables for quantitative study

Primary variables	Parameters
General and demographic data	Age
	Gender
	Individual fluoride concentration of drinking water (µg/ml)
Anthropometric data	Weight (g)
	Height (cm)
	Body Mass Index (kg/m^2)
Dietary data	Fluoride intake from each food and drink group (mg/d)
	Total fluoride intake from all drinks (mg/d)
	Total fluoride intake from all food (mg/d)
	Total dietary fluoride intake (mg/d)
	Prevalence of stunting

Primary variables	Parameters
	Prevalence of wasting
Tooth brushing habits	Frequency of brushing/day
	Type of used toothpaste and fluoride content (µg/g) of toothpaste
	Form of toothpaste
	Age of starting brush
	Brushing habit
	Person performing the brushing
	Person who puts toothpaste on tooth brush
	Fluoride concentration of toothpaste
Data on fluoride ingestion during tooth brushing	Weight of dispensed toothpaste (g)
	Amount of fluoride in dispensed toothpaste (mg)
	Amount of fluoride from toothpaste (mg/brushing)
	Total fluoride ingested from toothpaste (mg/brushing)
	Total fluoride ingested from toothpaste (mg/d)
	Total daily fluoride intake (Diet + toothpaste) (mg/d and mg/kg bw/d)
24-h urinary data	Urine volume (ml/d)
	Corrected urine volume for 24-h (ml/24 h)
	Fluoride concentration (µg/ml)
	Urinary fluoride excretion (mg/d and mg/kg bw/d)
Fingernail fluoride data	Weight of fingernail before cleaning (mg)
	Weight of fingernail after cleaning (mg)

Primary variables	Parameters
	Weight loss (mg)
	Fluoride concentration of fingernail (µg/g)
Toenail fluoride	Weight of toenail before cleaning (mg)
	Weight of toenail after cleaning (mg)
	Weight loss (mg)
	Fluoride concentration of toenail (µg/g)
Hair fluoride data	Fluoride concentration of hair (µg/g)
Saliva fluoride concentration	Fluoride concentration of saliva (µg/ml and µmol/l)
Plasma fluoride data	Fluoride concentration of plasma (µg/ml and µmol/l)
Assessment of completeness of 24-h urine	Urine volume (ml/24 h)
	Urine flow rate (ml/h)
Biomarker acceptability data (Questionnaire)	Attitude towards using the biological markers
	Perceived ease of collection of the biological markers
	Behavioral interest to the use of the biomarkers

4.8 STATISTICAL ANALYSIS

Data obtained were analysed using SPSS version 22, as summarised in Table 4.3. Ultimately, the samples were compared using an ANOVA.

Table 4.3: Summarised statistical analyses

STUDY	AIMS AND OBJECTIVES	STATISTICAL ANALYSIS
Study 1	Aim	Refer to the objectives
	Subsidiary aim	Descriptive statistics
	Objective 1	Descriptive statistics
	Objective 2	Descriptive statistics
	Objective 3	Descriptive statistics
Study 2	Aim 1	Descriptive statistics
	Aim 2	Refer to objectives 3 and 4
	Objective 1	Descriptive statistics
		T test
	Objective 2	Descriptive statistics
	Objective 3	Pearson correlation
	Objective 4	Anova post hoc test
	Objective 5	Pearson correlations
	Objective 6	Descriptive statistics
	Objective 7	Descriptive statistics
Study 3	Aim	No statistical analysis

4.9 VALIDATION OF ANALYTICAL METHODS, DIETARY ASSESSMENT METHOD AND COMPLETENESS OF 24-H URINE COLLECTIONS.

4.9.1 Fluoride determination

The reliability of the fluoride analytical methods was examined by re-analysing 10% of all samples for fluoride concentration. Samples of urine, nails (fingernails and toenails), hair, plasma and saliva (n = 72) selected randomly were re-examined using the same method of analysis.

The validity of the fluoride analytical methods was also examined by adding a known amount of fluoride to the same samples as mentioned in previous sentence. The

concentration of fluoride was determined in the samples with and without standards and used to investigate the validity of the analytical method.

4.9.2 Completeness of 24-h urines

The completeness of 24-h urine was examined by calculating the urinary flow rate of samples. Urinary flow rate is regarded as one of the markers for validation of completeness of urine and has been used by several authors based on WHO recommendations (Ketley and Lennon, 2001; Ketley *et al*., 2004; Maguire *et al*., 2007). WHO suggested flow rates are 5-160 ml/h and 9-300 ml/h for children (<6 years old) and adults (≥6 years old) respectively. In addition to the above, urine sample volumes <140 ml/24hr or more than 1200 ml/24hr should be discarded for children and urine samples <200 ml/24hr or more than 3000 ml/24hr should be discarded for adults (Table 4.6). Based on the above recommendations, samples of 24-h urine that do not meet the WHO criteria for children and adults were discarded.

Table 4.4 Recommended normal urinary flow rate by WHO (1999)

	Lower limit	Typical value	Upper limit
Urine volume			
<6 years (ml/24h)	140	500	1200
≥6 years (ml/24h)	200	1200	3000
Urine flow			
<6 years (ml/h)	5	20	160
≥6 years (ml/h)	9	50	300

CHAPTER 5: STUDY ONE: ASSESSMENT OF ACCEPTABILITY AND RELIABILITY OF BIOLOGICAL MARKERS OF EXPOSURE TO FLUORIDE IN THE UNITED KINGDOM

5.1 INTRODUCTION

This chapter is divided into three main sections, including: detailed methodology, qualitative study and quantitative study. This was then followed by an overall conclusion of both the quantitative and qualitative study.

5.2 AIMS AND OBJECTIVES

5.2.1 Aim and Subsidiary aim

The main aim was to investigate the preference for biological markers of fluoride exposure among two different age groups in the UK. The subsidiary aim was to evaluate the fluoride concentration of the biological markers.

5.2.2 Objectives

- Investigate the most acceptable biomarker of fluoride exposure by age group (4-5 and ≥20 years) using questionnaires.
- Evaluate the feasibility of collecting biological markers of fluoride exposure by age group (4-5 and ≥20 years).
- Evaluate the willingness of participants among the age groups (4-5 and ≥20 years) to provide any of the biological markers.
- Measure and compare fluoride concentration of different biomarkers of fluoride exposure.

5.3 MATERIALS AND METHODS

5.3.1 Study design

This study used both qualitative and quantitative approaches to address different aims and objectives. The study was carried out in two phases. Part A involved the administration of a questionnaire to the volunteers who accepted to take part in the research. Part B involved participants who were willing to provide any of the human biological samples to evaluate the concentration of fluoride in the samples.

5.3.2 Ethical consideration and approval

5.3.2.1 Ethical approval

Favourable ethics approvals were obtained from relevant Ethics Committee. Refer to section 4.3.1 (Page 71). Copies of the approvals are presented in Appendix 1 and 3 respectively.

5.3.2.2 Permission from parents or legal guardian/staff of University

- ***Qualitative study:*** With the assistance of the head teachers and deans in the primary schools and University respectively, questionnaires were circulated among the targeted participants. Completion of the questionnaire as either hard copy or online was regarded as consent for the study.
- ***Quantitative study:*** Participants who were willing to participate in the study were invited to a meeting at a convenient place where the details of the research were communicated to them by the researcher and all questions they might have were answered. They were given sufficient time to consider taking part prior to acceptance of study. All participants who were happy to take part completed the consent form for themselves and on behalf of their children prior to the commencement of the study and were told they could withdraw from the study without any explanation or reason.

5.3.3 Study location

This study was conducted in Middlesbrough and Newcastle, located in the Northeast of England (Figure 5.1). Middlesbrough is a large industrial town situated on the south bank of the river Tees with a population of approximately 138,400 receiving non-fluoridated water (0.15 mgF/l). Newcastle is located on the north bank of the River Tyne with a population of approximately 289,945 receiving optimally (artificially) fluoridated water (0.8-1.0 mgF/l).

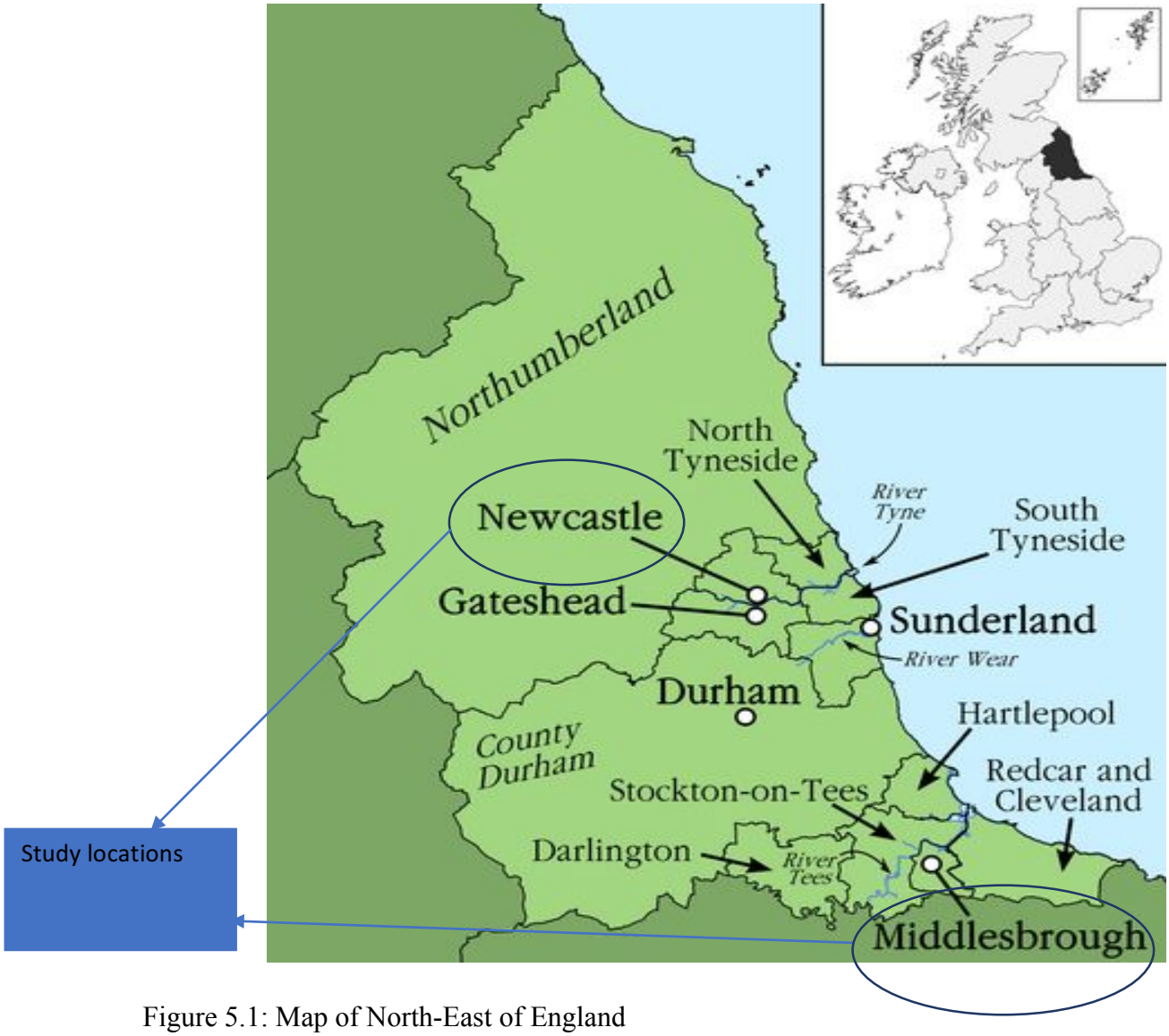

Figure 5.1: Map of North-East of England

Source: http://research.ncl.ac.uk/decte/toon/assets/images/NEMap.jpg

5.3.4 Self-reported questionnaire

5.3.4.1 Development of self-reported questionnaire

Two self-reported questionnaires (one for adults and one for children) were developed to measure the level of acceptability and feasibility of collecting the biological markers of fluoride exposure in children and adults. The questionnaires contained general demographic information collected using standardised categories/criteria including year of birth, ethnicity, religion, gender, postcode sector of residence of participant including adults and children, as well as specific bespoke questions related to the acceptability and feasibility of

collecting fluoride exposure biomarkers. Both questionnaires were developed by combining two standard scales for measuring acceptability and feasibility in children and adults and then augmenting them with a small number of questions relating to biographical details. The two standard scales included the Graphic rating scale and the Likert scale. The questionnaire was designed considering a proposed Technology Acceptance Model (TAM guide) on the theory of reasoned action, which has been used to broadly predict and explain human behavior in consumer adoption. Abdullah *et al*. (2016) identified five factors that influence acceptance from the TAM: perceived ease of use of a system, perceived usefulness, attitude towards using, behavioral intentional use, and actual system use. Behavioral intention to use is defined as a user's interest in using the system in the future. Perceived usefulness is defined as the degree to which a person believes that using a health intervention will enhance his or her health status, while perceived ease of use is defined as the degree to which a person believes that using an intervention would be easy. Perceived ease of use has a direct effect on perceived usefulness, and in turn would affect behavioral intention to use (Abdullah *et al*., 2016).

Perceived usefulness and perceived ease of use were used to design the Biomarker Acceptability Questionnaire (BAQ). Participants were asked how they felt about having to collect their own biological samples. Responses on each of the markers were given separately and were based on a Likert scale where participants were required to answer ranging from 'totally unacceptable' to 'perfectly acceptable'. The perceived ease of use was investigated by asking participants to respond using a graphical rating scale how they would find the collection of the biological marker. In the same way responses to each biological marker were provided separately. The behavioral intention to use was investigated by a 'yes' or 'no' response and a scale of 1-6 was used for participants to comment on which of the biomarkers they least preferred to most preferred among all the biological markers.

Other questions that were investigated, including the socio-demographic details, were extracted from standard questionnaires that have already been validated from Scottish and English surveys (Census programme, 2011; Office of National Statistics, 2015), since attitude to the acceptability of using the biological markers is also considered.

5.3.4.2 Pre-testing of self-reported questionnaires

The questionnaires were informally pre-tested on a small sample of colleagues (and their children) at the Institute of Health and Social Care, Teesside University to establish the

feasibility, applicability, and acceptability of the questionnaire among the two age groups, although the questions used in the questionnaire were obtained from standardized instruments which were known to be valid and reliable. Ten participants volunteered to complete the pre-test survey and the results showed that the questionnaire was feasible, applicable and acceptable to use within the population. However, some adaptations were made on the instructions relating to the collection of the biomarker samples in the questionnaire to enhance clarity.

5.3.4.3 Online survey

After the self-reported questionnaire had been tested and corrected for error, the online survey was then created using the Bristol Online Survey (BOS) tool. A date and time question was used to determine the age of participants, while a selection list question was used for the ethnicity information as well as the religion of the participant. Scale and rank questions on the survey tool were used to determine the acceptability, feasibility, and validity of the biomarkers. Pictures of the techniques for collecting the biomarkers were added to make the survey interesting for participants. A file containing an opportunity to participate in the quantitative study was added to the survey for those participants who were willing to take part. The survey was structured into two sections (demographic section and acceptability as well as the feasibility of collecting fluoride exposure biomarkers), and the contact details of the researcher were added to the contact and copyright section of the BOS should the participant wish to reach the researcher. Permission was then obtained from the Graduate Research School, Teesside University to use the University logo to customise the appearance of the survey and to give authority. Upon approval, this was used to customize the appearance of the survey. Prior to launching the survey, the minimum and the maximum number of participants were added and the date for the completion of the survey. After launching, the link to the online survey was copied and pasted into the correspondence email scheduled for the participants.

5.3.5 Subjects

The subjects were children and adults of both genders.

1. Age: Children and adult with ages 4-5 and ≥20 respectively were recruited. The date of birth of children and adults was obtained from the response to the questionnaires completed by the participants.

2. Gender: The study was designed to include both males and females in order to identify any possible differences in the level of acceptability and feasibility of obtaining biological markers from participants as well as any differences in metabolism of fluoride for participants willing to provide any of urine, hair, nails (fingernail and toenail), saliva and blood, associated with gender.

3. Social class: Participants were targeted from all social classes to determine their differences in the level of acceptability of the different biomarkers of fluoride exposure (see Appendix 24).

5.3.6 Sample size

The quantitative part of the study involved a total sample of 220 participants which was equally distributed among adults (n=110) and children (n=110). The sample size was estimated based on differences in the mean value of acceptability resulting from the two independent age groups and on the level of acceptability for the collection of the biomarkers (detailed information in chapter 4, section 4.3.4).

5.3.7 Recruitment

Two routes of recruitment were considered to approach potential participants for this study: 1) school children and their parents and 2) university staff and their children.

5.3.7.1 Recruitment through primary schools:

Recruitment of Middlesbrough schools: Since the level of acceptance and suitability of the biomarkers could vary amongst different social and educational classes, subjects were selected from high and low social areas in order to measure any difference in acceptability of the biomarkers among the different socio-economic groups. Based on the Middlesbrough Council website, the borough is divided into 23 wards. According to the Index of Multiple Deprivation (IMD) 2010 for Middlesbrough wards, the wards were aggregated into five bands. Band 1 was the most deprived 10%, band 2 was the next most deprived 10-20% wards, band 3 was the next most deprived 20-40%, band 4 was the next most deprived 40-60% and band 5 was the least deprived 0-40% ward (as shown in Appendix 24 for most recent (2015) classification). Information on the number of primary schools and their distribution within the three bands were obtained (as shown in Table 5.1). Among the 46 primary schools in Middlesbrough, 11 primary schools were selected randomly from bands 1, 2, 3 proportional to the number of schools in each band. However, any selected school

not willing to participate was dropped and another selected using the same set of random numbers (see Table 5.2).

Recruitment of children and parents: Schools were contacted by phone or email, inviting them to participate in the research. They were given time to consider whether their school would take part in the study and were followed up by phone/email for any expression of interest. Upon agreement by the head teacher of selected schools to participate, the researcher (Mr. Idowu) visited the school and gave out study packs (containing questionnaire, invitation letter and response form for participation in future studies) to the head teacher of the school to pass them to the parents of the children. The researcher answered all questions that the head teacher might have before passing on the study packs. All children aged 4 – 5 years attending the selected primary schools and their parents were eligible to take part. Arrangements were made with the head teacher on when the completed questionnaires would be picked up by the researcher in the school. Once a school declined, the same random method used in selecting that school was adopted to select another school.

5.3.7.2 Recruitment through university:

The staff of Teesside and Newcastle Universities and/or their children were approached through emails. The email contained an invitation and introduction to the study as well as the eligibility criteria. A link to the online survey was also attached to the email for participants who were happy to take part in the study to complete. An attachment of a softcopy of the questionnaire and opportunity for future research (Response form) was also added

Table 5.1: Index of Multiple Deprivation (IMD) 2010 for Middlesbrough wards and distribution of schools

Ward (N E S W)	2010 IMD Ward Rank	2010 most deprived (%)	No of schools
Middlehaven	6	1%	1
Thorntree	15	1%	2
North Ormesby and Brambles	41	1%	3
Pallister	65	1%	5
Beechwood	67	1%	4
Park End	71	1%	2

Ward (N E S W)	2010 IMD Ward Rank	2010 most deprived (%)	No of schools
Gresham	100	3%	3
University	103	3%	2
Clairville	144	3%	2
Beckfield	281	5%	3
Ayresome	285	5%	2
Hemlington	341	5%	2
Park	779	10%	-
Ladgate	1239	20%	4
Stainton & Thornton	1675	30%	1
Coulby Newham	1863	30%	4
Marton West	6786	30%	1
Marton	5283	30%	1
Linthorpe	3301	>30%	3
Brookfield	5074	>30%	2
Kader	5341	>30%	2
Acklam	5486	>30%	2
Nunthorpe	7337	>30%	3

5.3.8 Qualitative study: procedure

5.3.8.1 Subjects

The only inclusion criteria for participants to be eligible to take part in the study was being aged 4-5 or ≥20 years old. A summary of the procedure is illustrated in Figure 5.2.

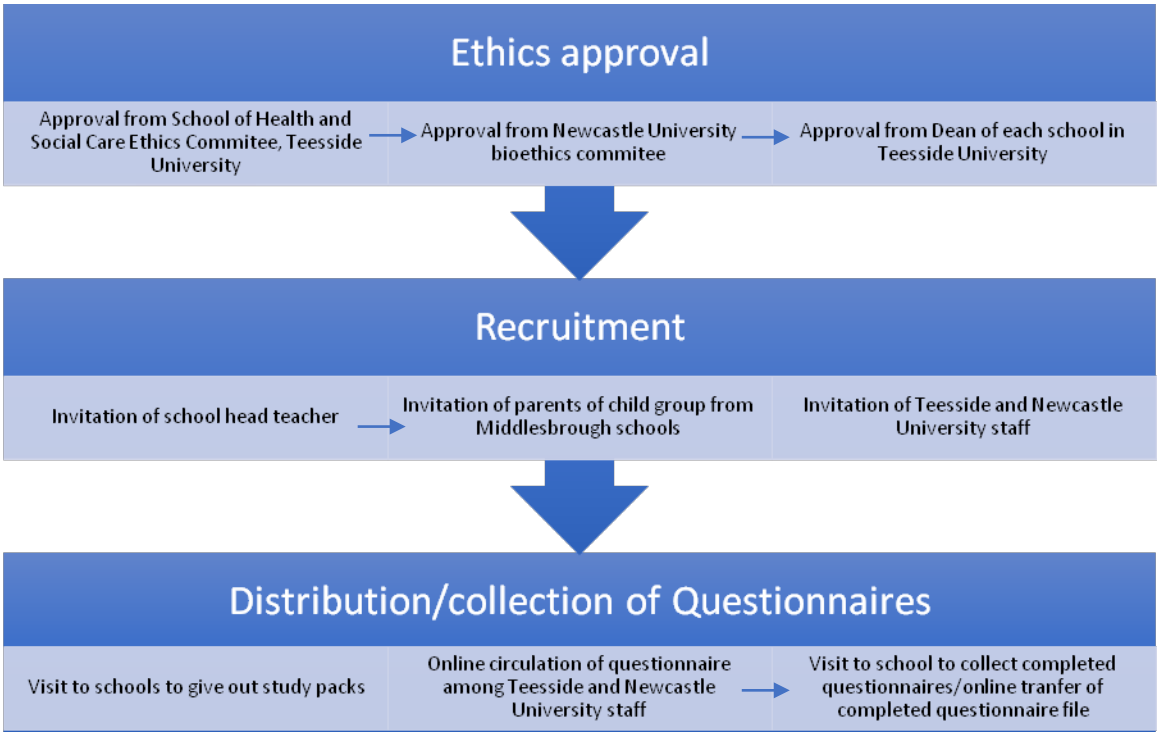

Figure 5.2 Study design of qualitative study

5.3.8.2 Hard copy questionnaire

Each study pack contained a questionnaire for the parent to complete for themselves and another to complete on behalf of their child (see Appendix 16 and 17), an Invitation letter (see Appendix 6) and a response form (see Appendix 6). Parents were not obliged to complete the questionnaire. However, parents who completed the questionnaire were assumed to have consented to the study. Instructions regarding the completion of the questionnaire were included in the questionnaire and participants were assured that the answers provided would not be linked to them or the school participating in the study. On the arranged date, the researcher visited the school and picked up the completed questionnaires from the head teacher.

5.3.8.3 Soft copy questionnaire

Upon receipt of the email, those who were willing to participate completed the survey online for themselves and/or their 4-5 years old child and others completed the attached soft copy and returned it to the researcher via email. The identity of the participants was anonymous as no name or any means of identification was written in the questionnaire.

5.3.9 Quantitative study: procedure

5.3.9.1 Participants

All those who participated in the qualitative study were invited to take part in this study as well. To be eligible to take part in this study, the participants should have met the following inclusion criteria: Between 4 - 5 or ≥ 20 years old of either gender, with no dietary restriction, no chronic or metabolic disease and urinary infection, no oral disease (no tooth or gum pain) and no professional dental treatment such as the use of fluoride varnish or filled tooth for at least three months prior to the start of the study, had parental consent (for children), ability and availability to provide a 10 ml of blood (participant willing to provide blood).

However, those who met any of the following criteria were excluded from taking part in the study: using medication, had a restricted diet, had metabolic disease and urinary infection, had oral disease (tooth or gum pain) and with professional dental treatment such as the use of fluoride vanish for at least three months prior to the start of the study, had any nail disease (applicable for participants willing to give nail sample) and unwilling to stop using nail varnish for the specified period up to sample collection or unwilling to remove nail varnish for specified period up to sample collection, using hair dye, individuals with history of blood borne pathogen (e.g. viral hepatitis, HIV/AIDS) due to laboratory restriction (participants willing to provide blood sample).

The researcher gave each participant an informed consent form after the medical history form had been fully examined. The consent form was completed after careful consideration by the participant and stored in a safe cabinet by the researcher at Teesside University. Informed consent for children aged 4 –5 years old was also completed by the parent/guardian before the commencement of the study. All documents completed by the participants remained anonymous as no name or means of identification was written.

5.3.9.2 Recruitment

Children and parents: Participants who were happy to take part in the study, completed the response form included in the study pack. The researcher then visited the school to give the study packs containing study information, informed consent, personal data form, fluoride exposure questionnaire, abbreviated medical history form, to the head teacher to pass them to the selected children whose parents were happy to take part in the study as well as the parents themselves. The researcher then contacted the parents by phone to answer any

questions they might have and they also contacted the researcher for further clarification. After they had carefully considered the study and accepted to take part, they were contacted by phone and a visit was arranged at a convenient place which was home, child's school or Teesside University (School of Health and Social Care) where they were assessed for their health status using the medical form (see above for details).

The staff of Teesside and Newcastle University: For those who consented to the study (as discussed above), a visit was further arranged at a convenient place [home or Teesside University (School of Health and Social Care)].

5.3.9.3 Study procedure

Participants were not required to provide all the biological markers for estimation of fluoride exposure, which included: 24-h urine, whole saliva, blood, hair, and nails (fingernail and toenail), but chose any of the samples they were happy to provide. A visit was agreed when a Fluoride Exposure Questionnaire (FEQ) (see Appendix 15) and collection kits to use, depending on which of the biomarkers participants were willing to provide, were handed to the participants for use at home or wherever they deemed convenient. Each participant was provided with written instructions on how to collect the samples (see Appendix 20) and for completing the FEQ. After collection, participants arranged a meeting with the researcher in a convenient place (Teesside University, their home, or child's school) where the samples were picked up by the researcher. Samples of blood were collected by a qualified nurse (Lesley Cooper) on an arranged date at the School of Health and Social Care, Teesside University. A summary of the procedure is illustrated in Figure 5.3.

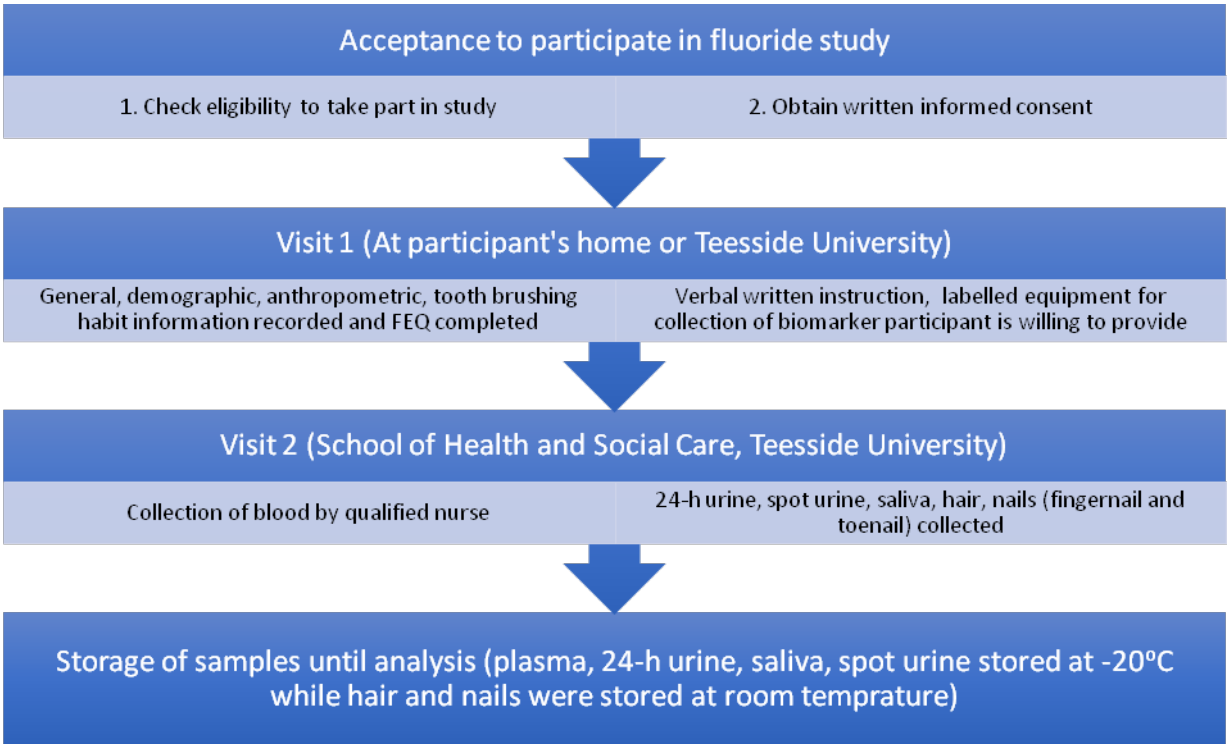

Figure 5.3: Study design for quantitative study

5.3.9.4 Data and sample collection

Each participant was assigned an identification number (ID) at the time of recruitment and the number was used throughout the study for anonymity and confidentiality. On the day of the (second) visit, participants provided the biomarker samples and completed FEQ as well as the demographic form (see details below). However, blood was only collected in the physiological lab, School of Health and Social Care, Teesside University by a qualified nurse (details discussed below).

Demographic data and anthropometric measurements: Demographic information of each participant, including: name, home address, date of birth, was recorded (see Appendix 15). The height and weight were measured in the physiotherapy laboratory of the School of Health and Social Care, Teesside University and recorded on the anthropometric data collection sheet during the first visit. Height was measured vertically without shoes to the nearest 0.5 cm and weight also measured simultaneously with the height to the nearest 0.1 kg using a Telescopic measuring rod for Seca column scales (Seca Mod. 220, Germany). These recorded values were used throughout the studies to avoid errors since samples of biomarkers were collected on this date.

Fluoride Exposure Questionnaire (FEQ): Parents/guardians of children as well as other adults recruited for the study completed the FEQ containing information about previous dental history, use of dental supplements and demographic information as well as a Food frequency questionnaire (FFQ). The FEQ employed was a modification of that used in the "IOWA fluoride study" (Levy *et al*. 2002), and previously validated by Marshal *et al*. (2003). The questionnaire was used to assess past fluoride exposure and present intake of toothpaste and fluoride supplement use.

Collection of biomarker samples: All biomarker samples were collected by the researcher on the second visit and the completed FEQ. Incomplete or incorrect answers were clarified with the participants. For children aged 4 – 5 years old, the researcher discussed details of the FEQ with their parents. A water sample (about 10 ml) from the participants' primary source of drinking water was collected and analyzed for fluoride (see Chapter 4 for details).

Fluoride Acceptability Questionnaire (FAQ): Participants who took part in the quantitative study completed a second fluoride acceptability questionnaire described in Section 4.6.4, after providing the biomarker sample(s) they were willing to give.

Transportation of samples: Blood and tissue samples collected were sealed inside a waterproof box and labelled properly (name of the sample, date of collection and identity code). The researcher ensured appropriate packing of the blood samples to absorb spillage if the vials leaked. Samples were taken to the fluoride laboratory at the School of Health and Social Care, Teesside University.

5.3.9.5 Sample preparation, storage, analysis and disposal

Samples were prepared and stored according to standard analytical procedures as described in chapter 4. Samples of urine and water were divided into two aliquots, one for analysis of fluoride and the other kept as back up. Other samples of saliva and blood were centrifuged to remove debris, proteins and separate the plasma respectively. Nail samples were cleaned up, oven dried and homogenized.

All samples were stored at -4^{o}C in the fluoride laboratory, School of Health and Social Care, Teesside University prior to analysis except samples of hair and nails (fingernail and toenail) which were kept at room temperature.

5.3.9.6 Sample analysis

Analytical methods of fluoride analysis of samples have been detailed in chapter 4.

5.3.9.7 Disposal of samples

Urine samples were disposed of in the allocated toilet. All saliva, blood, nail and hair samples collected were used for analysis. Bijous, containers, and bottles containing urine were placed in Virkon solution (1%) for two hours prior to disposal. The containers were rinsed and placed in yellow bags, labelled as clinical waste and disposed of according to Teesside University disposal systems. Disposable gloves and plastic aprons were put in clinical water bins (yellow, labelled bin liners) for incineration. Disposable plastics (tubes, pipette tips, culture mask etc.), disposable glassware and blood sample bottles were decontaminated and kept in clinical waste bins (yellow, labelled bin liners) for incineration. All blood soiled materials were also disposed of in a yellow clinical waste bin. Whenever there was excess human material (hair, nail, plasma), it was decontaminated and placed in clinical waste bins (yellow, labelled bin liners) for incineration.

5.3.10 Data management and handling

Data obtained were not traceable to the individual participant as data relating to the identity of the individual was not recorded on the questionnaire. The responses were collected in the questionnaire and the hard copy was kept in a file which was stored in a secured space within Teesside University and will be destroyed after six years.

Two categories of data were generated from this study: 1) data on acceptability and feasibility of using the biological markers of exposure to fluoride was generated from the questionnaire (FAQ); 2) intake and excretion data generated from the FEQ (Oral hygiene questionnaire and FFQ) as well as 24-h urine collection respectively and retention data generated from saliva, hair, and nail (fingernail and toenail) collections.

Qualitative study: Qualitative data were managed using the Statistical Package for Social Sciences Software (SPSS) version 22 (see details in chapter 4, section 4.6.1).

Quantitative study: Details of the quantitative data have been discussed in chapter 4.

5.3.11 Data Analysis

5.3.11.1 Qualitative data analysis

The survey was analysed using descriptive statistics to summarize the data set in terms of frequencies of categorical variables as well as the spread of continuous variables. Means were reported for the continuous variables and for categorical variables, percentages of the different categories were reported. Bar charts were also generated for the categorical variables. The demographic statistics were first described followed by other analyses which were done according to the objectives of the study. Yes/No questions were analyzed by Chi-square test.

5.3.11.2 Quantitative data analysis

All data generated from the study were entered in Microsoft Excel datasheets upon completion and a summary file containing the main variables was generated. Descriptive analysis was derived using the statistical package SPSS.

5.3.12 Quality assurance

The database needed to be checked for error and cleaned before analyses were conducted as inconsistencies could arise at any stage of data processing and management which could distort the findings.

Errors were minimized during data entry by using computers that were programmed to detect the error. Thereafter, simply an explanatory data analysis test was used to detect any inconsistent or incorrect data entered, e.g. for continuous variables, calculating range were used to identify if variables were in the expected range and, for categorical variables, a test for frequencies was run to identify categories of each of the variables.

5.4 RESULTS

The outcome variables generated for the present study are shown in Chapter 4.

5.4.1 Qualitative study

5.4.1.1 Response and attrition rates

Table 5.2 shows the response and attrition from schools in Middlesbrough. Ten schools were selected randomly to take part in the study until all 46 schools were contacted since the

school head teachers of most of the randomly selected schools declined participation in the study. In all, three schools agreed to take part in the study.

A total of 120 questionnaires were sent to the interested head teachers to pass to the parents. Out of these, 5 questionnaires were completed by the parents representing 4% of the number sent out.

Online: A total of 125 participants completed the questionnaire, including 104 adults and 21 questionnaires completed by parents on behalf of their child (Figure 5.4). This represented a response rate of 61% overall. However, a 99% response rate was obtained from the adult survey while only 23% completed the survey on behalf of their child.

Table 5.2: School recruitment information

Band	Total primary schools (No)	Participants required (%)	Number of participants	Primary schools Accepted (No)	No of Q. Dispatched	Completed Questionnaires
1	14	30.4	33	Nil	Nil	Nil
2	12	26.1	29	1	45	Nil
3	20	43.5	48	2	75	5
All	46	100	110	3	120	5

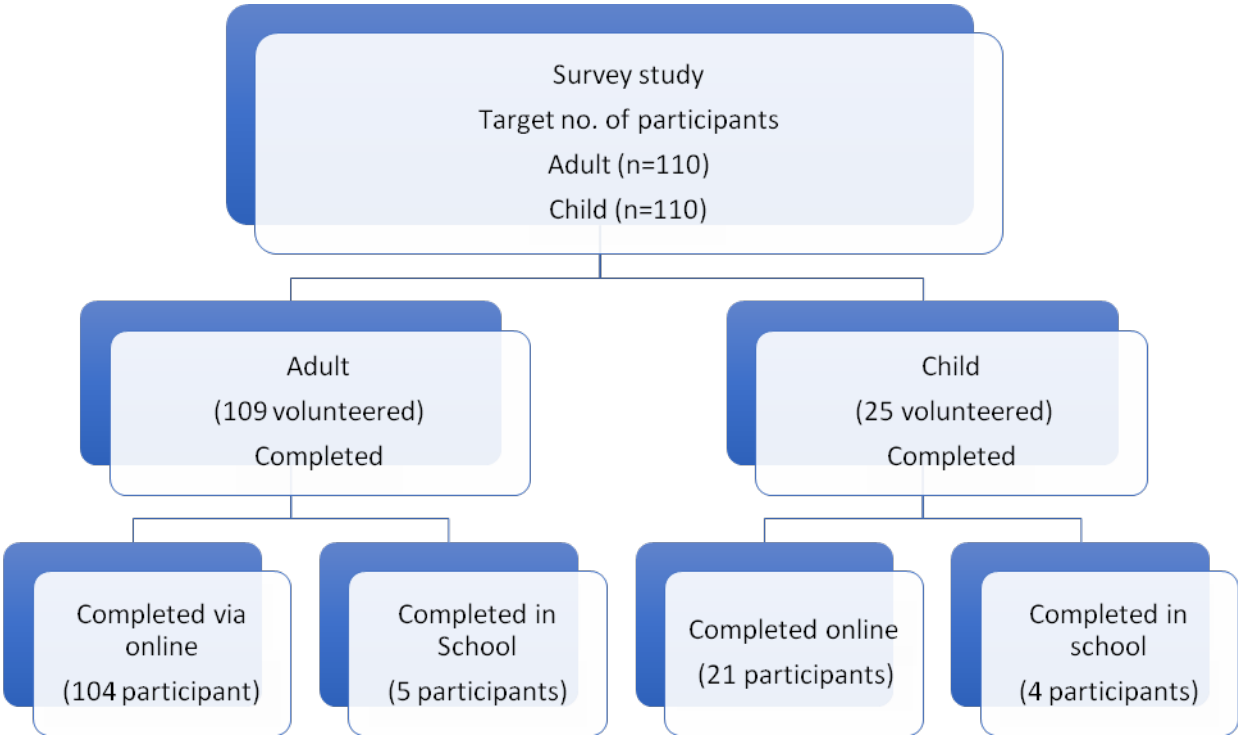

Figure 5.4: Summary of target number and completed number

5.4.1.2 Socio-demographic characteristics of participants

- **Adult age group**

This is from the survey completed by adults exclusively. Table 5.3 illustrates the socio-demographic data of adults who completed the questionnaires from Teesside and Newcastle University as well as parents of children from Middleborough schools. The results show that more females participated in the survey than males with a total of 64.2% (n=68) females and 35.8% (n=38) males. There was a wide distribution across the age groups 18-24, 25-34, 35-44, 45-54, ≥55 with percentages 8.7%, 24.3%, 32.0%, 17.5% and 17.5% respectively. In total, 84.4% (n=87) of the 105 participants who completed the survey had a job. However, two of the participants did not complete their employment status. A total of 81.1% (n=86) were educated to degree level while the remaining few were lower than degree level. Majority of participants who completed the survey were white (62.1%) or black (33.0%), while a small percentage (4.9%) was Asians. The highest percentage of adults was Christians (61.7%).

Table 5.3: Socio-demographic characteristics of adults

		Frequency	Percentage (%)
Gender	Male	38	35.8
	Female	68	64.2
Age range	18-24	9	8.7
	25-34	25	24.3
	35-44	33	32.0
	45-54	18	17.5
	More than 55	18	17.5
Ethnic background	Black	34	33.0
	Asian/Chinese	5	4.9
	White	64	62.1
Do you have a job?	Yes	87	84.4
	No	16	15.5
Highest level of education	GCSEs/O-levels	4	3.7
	A-levels/Diploma	12	11.3
	University degree	86	81.1
	Vocational qualification	2	1.9
	No qualification	1	0.9
Religion	No religion	32	29.9
	Muslim	3	2.8
	Christian	66	61.7
	Hindu	1	0.9
	Jewish	1	0.9
	Others	4	3.7

- **Child group**

This is from the survey completed by parents on behalf of their children. Table 5.4 shows the socio-demographic characteristics of parents/guardians of children aged 4-5 years who participated in the survey. The mean age of children whose parents completed the survey was 4.3 years. Majority of children who participated were females (59.1%). Most of the participants were white (78.8%) and blacks constitute 16.7% and the remaining 8.3% were Asians. Only 3 (12.6%) of the parents did not have a job, while 8.3% would prefer not to let their job be known. Eighty-one percent of the respondents had completed a university degree. In total, 58.3% of those who completed the survey were Christians while 37.5% have no religion.

Table 5.4: Socio-demographic characteristic of child's parents

		Frequency	Percentage (%)
Ethnic background	Black	4	16.7
	Asian/Chinese	2	8.3
	White	17	70.8
Does parent have a job (parent completing the form)?	Yes	19	79.2
	No	3	12.5
Highest level of education of parent (completing the form)	GCSEs/O-levels	0	0
	A-levels/Diploma	2	9.1
	University degree	18	81.8
	Vocational qualification	2	9.1
	No qualification	0	0
Religion	No religion	9	37.5
	Muslim	1	4.2
	Christian	14	58.3

5.4.1.3 Attitude, perceived ease and behavioural interest toward using the biological markers of exposure to fluoride

A) Attitude towards using the biological markers

- **Adults**

There was low variability between acceptance and unacceptance of the collection of 24-h urine as a biological marker of exposure. The percentages were comparable: 37% of the participants found 24-h urine perfectly acceptable and 22% found it totally unacceptable. However, 10% and 17% found it unacceptable and slightly unacceptable respectively. Therefore, considering the attitude of the participants towards 24-h urine, 49% of participants reported that they found the collection of 24-h urine "totally unacceptable", "unacceptable" or "slightly unacceptable", while 40% said they found its collection "perfectly acceptable" or "slightly acceptable" (Figure 5.5). In contrast, when the same participants were asked how they felt about the way spot urine was collected, 60% found it perfectly acceptable or slightly acceptable while 25% found the collection either totally unacceptable, unacceptable or slightly unacceptable. There was a slight drop to 47.5% of those who found two day spot urine perfectly acceptable or slightly acceptable when they were asked if they could do the spot urine sample collection for a two-day period and a corresponding increase to 37% of participant who found two-day spot urine collection totally unacceptable, unacceptable or slightly unacceptable (Figure 5.6).

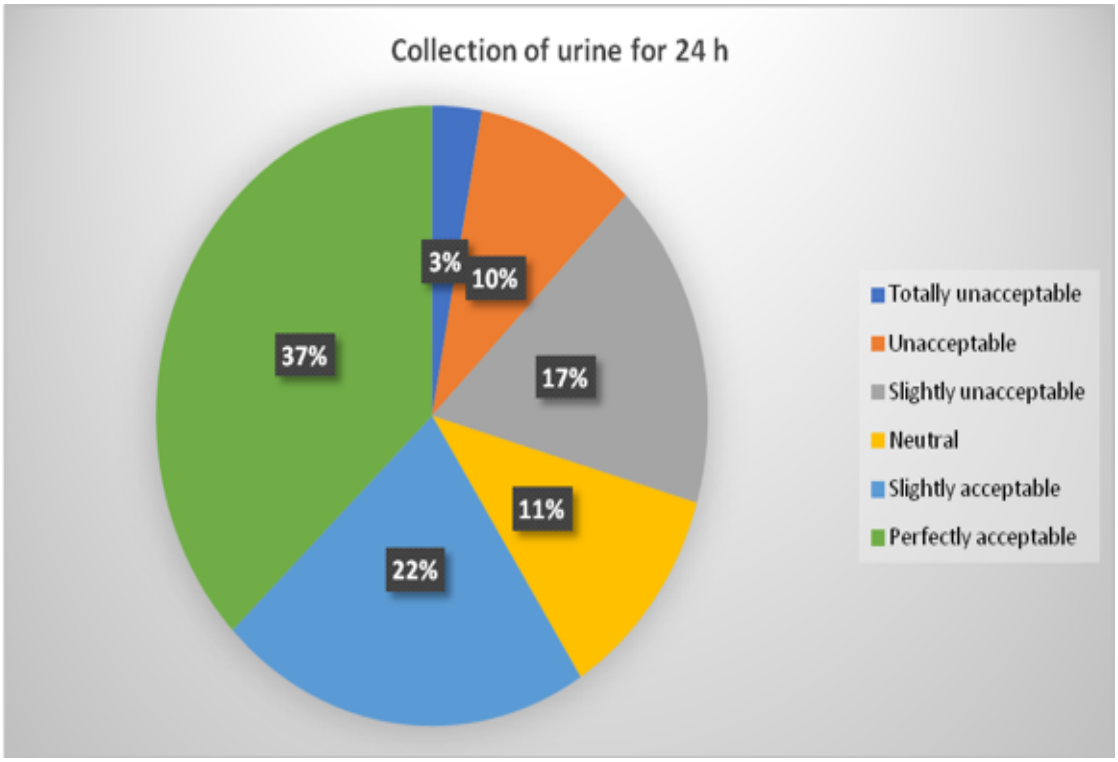

Figure 5.5: What do you feel about having to collect your urine for 24 h?

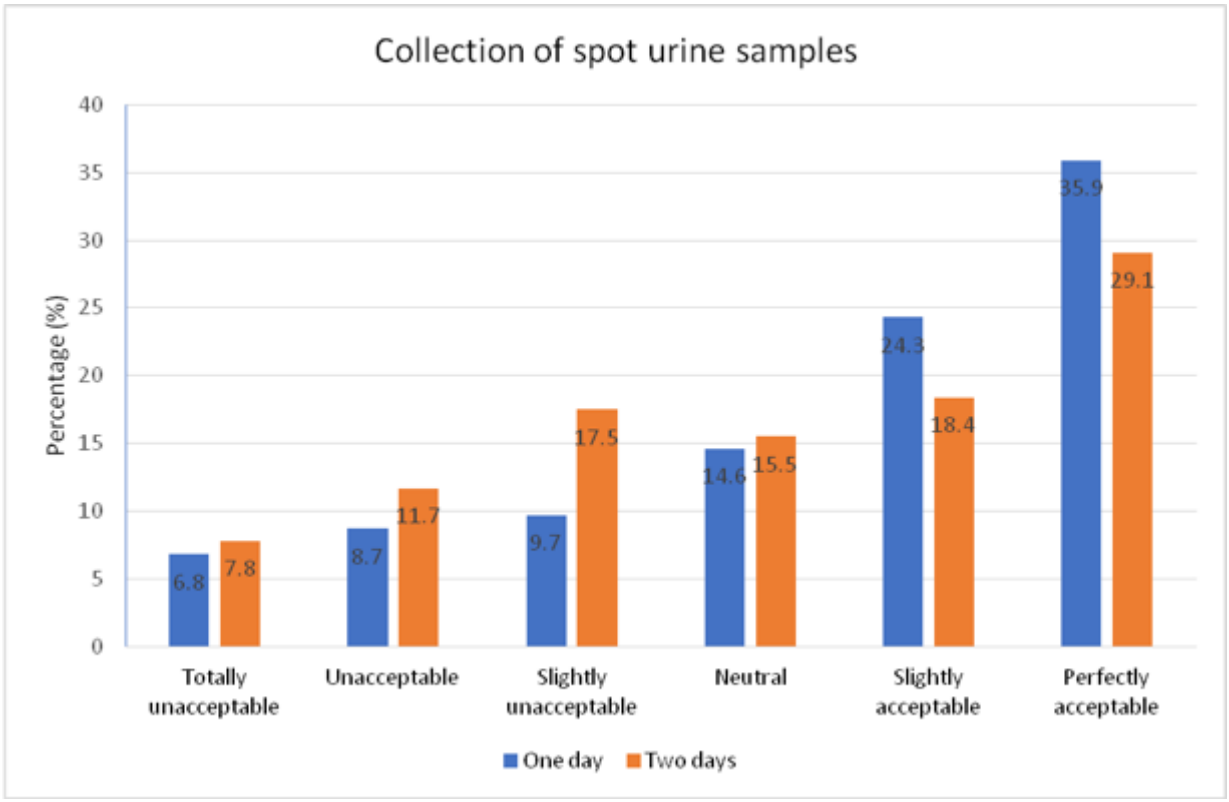

Figure 5.6: What do you feel about having to collect spot urine samples?

Contrary to how participants felt about the collection of 24-h urine samples for estimation of exposure to fluoride, which is the most commonly reported biomarker, 46% found saliva collection perfectly acceptable while 35% found the collection either totally unacceptable, unacceptable or slightly unacceptable (Figure 5.7). Though 49% felt saliva collection is totally acceptable or slightly acceptable, an even greater number, 60% preferred collection of blood for the estimation of fluoride exposure. Only 28% of the respondents felt the collection of blood was either totally unacceptable, unacceptable or slightly unacceptable (Figure 5.8).

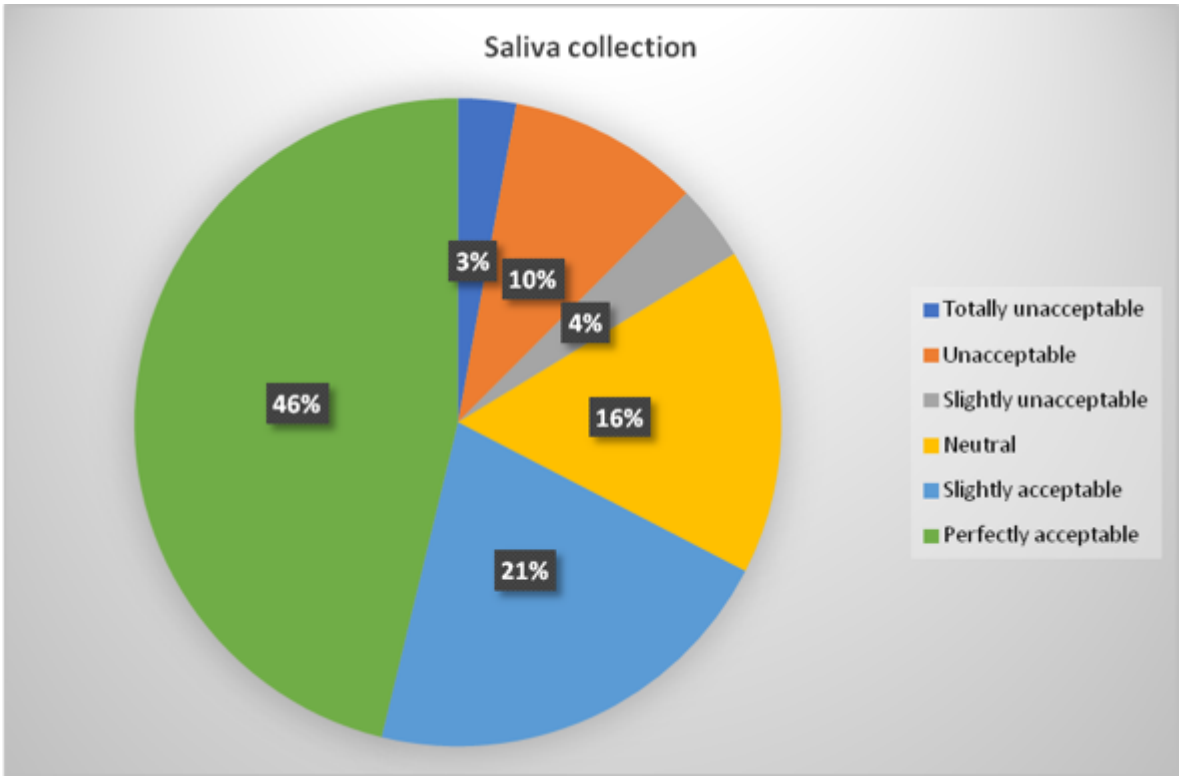

Figure 5.7: What do you feel about the saliva collection?

Conversely, 57% of respondents found the collection of nails, which are a long-term marker of exposure to fluoride, to be perfectly acceptable and a combined 71% of those surveyed said they found the collection perfectly acceptable or slightly acceptable while 18% found it totally unacceptable, unacceptable or slightly unacceptable (Figure 5.9). Meanwhile, a combined 60% felt that the way hair samples would be collected was totally unacceptable,

unacceptable or slightly unacceptable. Thirty-three percent found the way hair would be collected to be totally acceptable or slightly acceptable (Figure 5.10).

Overall, a combined 59%, 60%, 48%, 67%, 71%, 71% and 33% found the way samples of 24-h urine, spot urine (one day), spot urine (two day), saliva, blood, nails and hair, respectively, would be collected perfectly acceptable or slightly acceptable while 30%, 25%, 37%, 16%, 17%, 18% and 53%, respectively, would find the biological markers' collection totally unacceptable, unacceptable or slightly unacceptable.

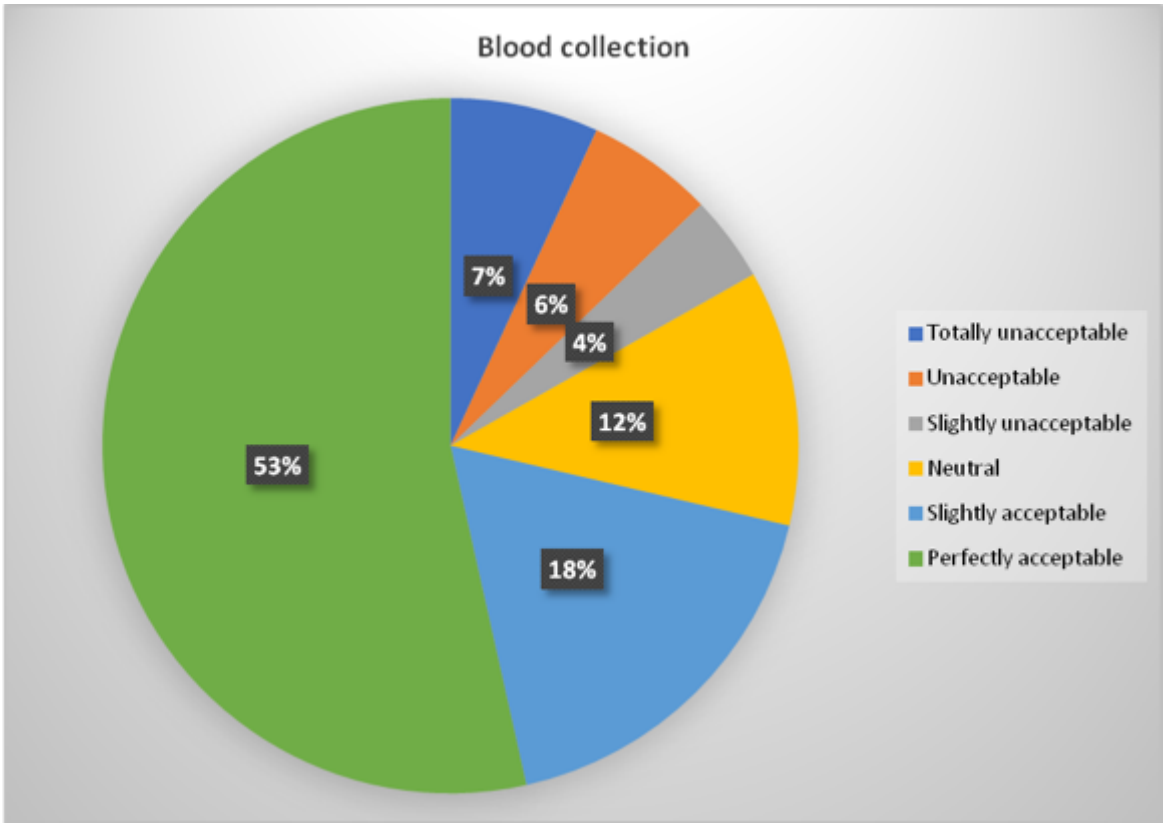

Figure 5.8: How do you feel about the way blood is taken?

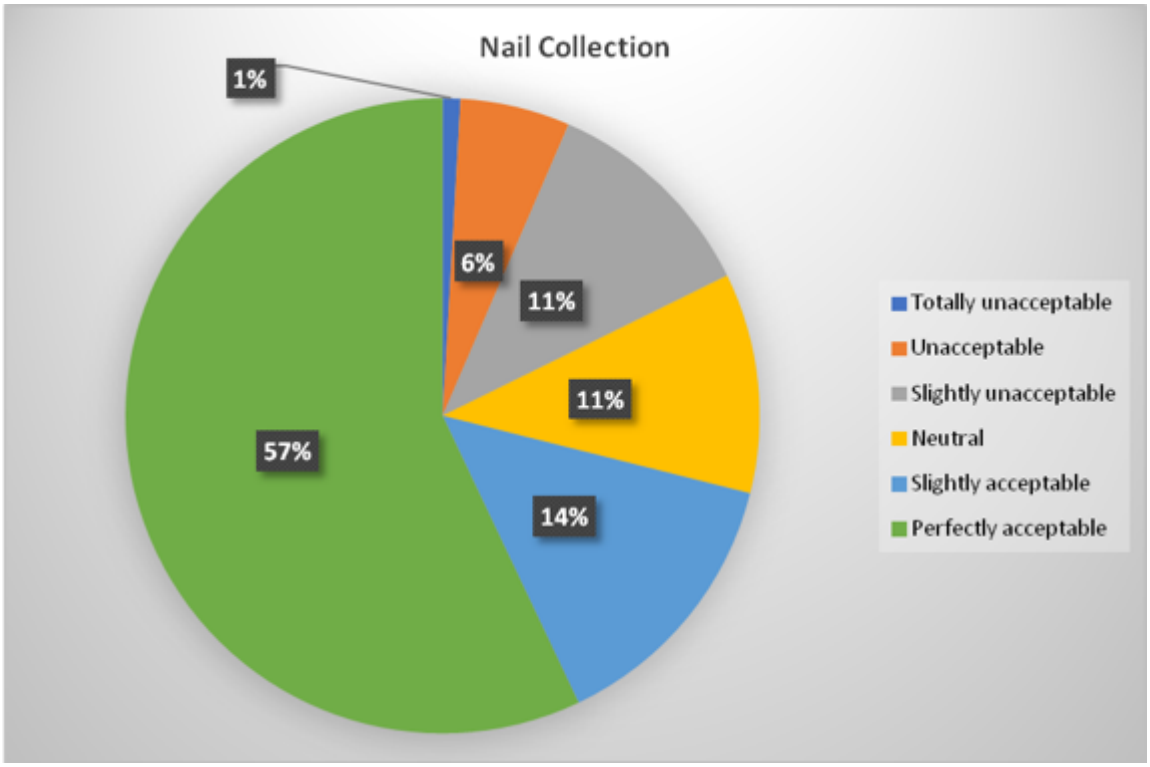

Figure 5.9: What do you think about being asked to collect your nails?

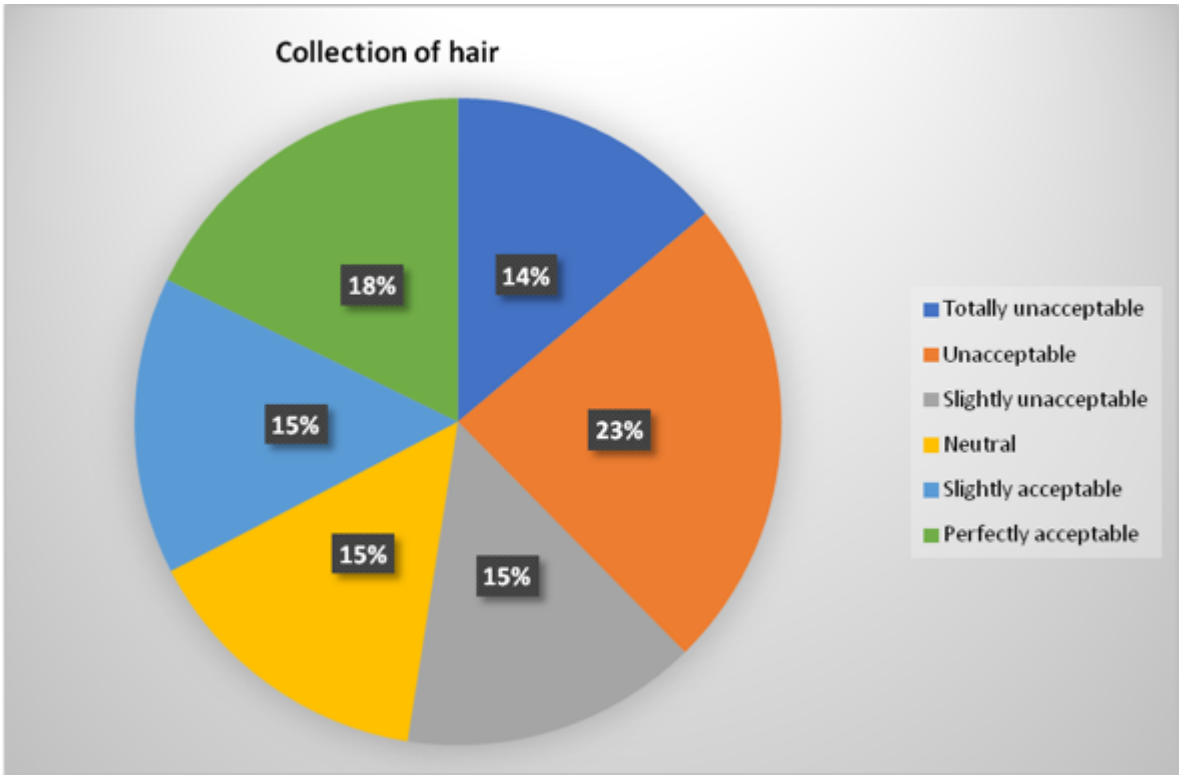

Figure 5.10: What do you think about being asked to collect your hair?

- **Children (Perception of parents about collection of biomarkers from children)**

These findings are less reliable/less valid than the corresponding findings for adults because of the small number of subjects (25 participants) who participated in the survey. This is 25% of the required number of participants which is less representative based on the required sample size.

Overall, 37% of the respondents found the collection of child urine for estimating exposure to fluoride perfectly acceptable or slightly acceptable, while 42% found it totally unacceptable, unacceptable or slightly unacceptable (Figure 5.11). However, when asked if their child could provide one-day spot urine samples, 52.2% found it perfectly acceptable or slightly acceptable, although, there was a drop in the percentage (42.8%) when the collection of spot urine samples was extended to a two-day period. A total of 38% of the participants found the two-day spot urine sample totally unacceptable, unacceptable or slightly unacceptable (Figure 5.12).

In total, 50% of participants found the collection of saliva for the estimation of exposure to fluoride to be perfectly acceptable or slightly acceptable while 29.3% found its collection totally unacceptable, unacceptable or slightly unacceptable (Figure 5.13). Conversely, 50% of the participants felt the collection of blood from their child to be totally unacceptable, unacceptable or slightly unacceptable, 38% found it to be perfectly acceptable or slightly unacceptable, while 12% had mixed feelings regarding its acceptability for use in estimation of exposure to fluoride (Figure 5.14).

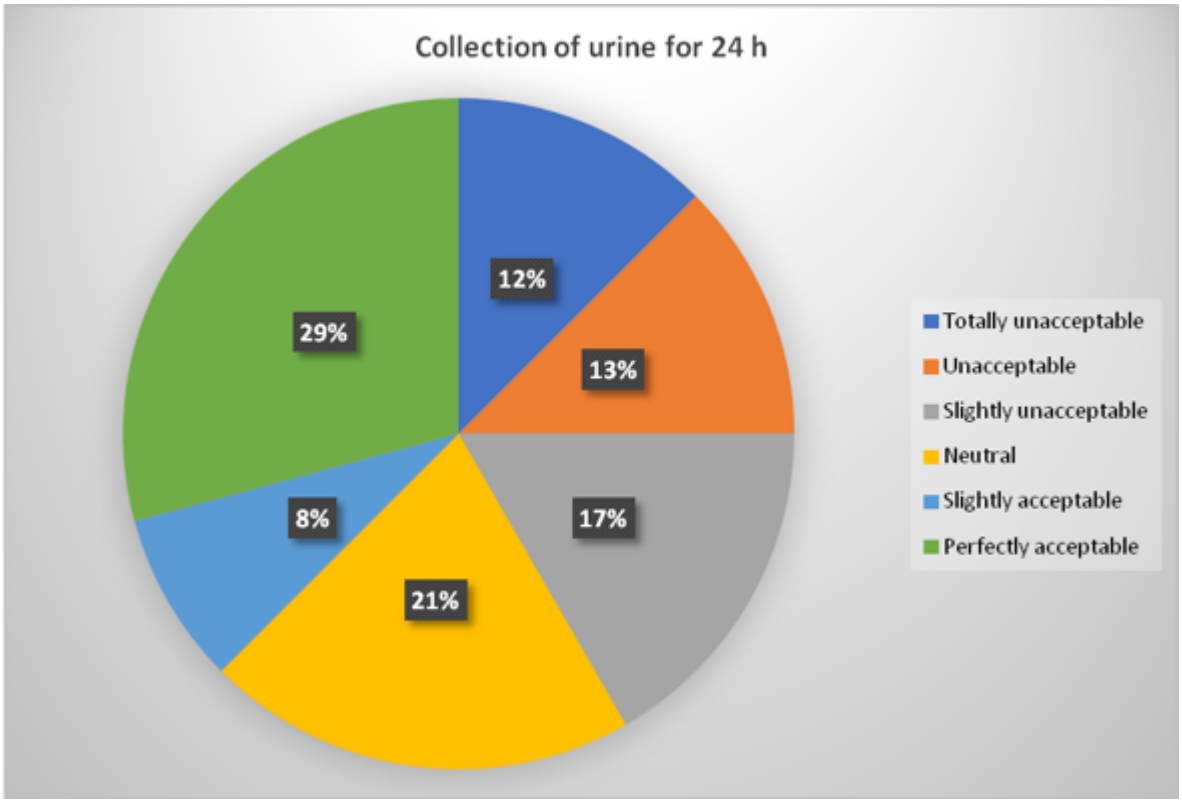

Figure 5.11: what do you feel about having to collect your child's urine for 24-hrs?

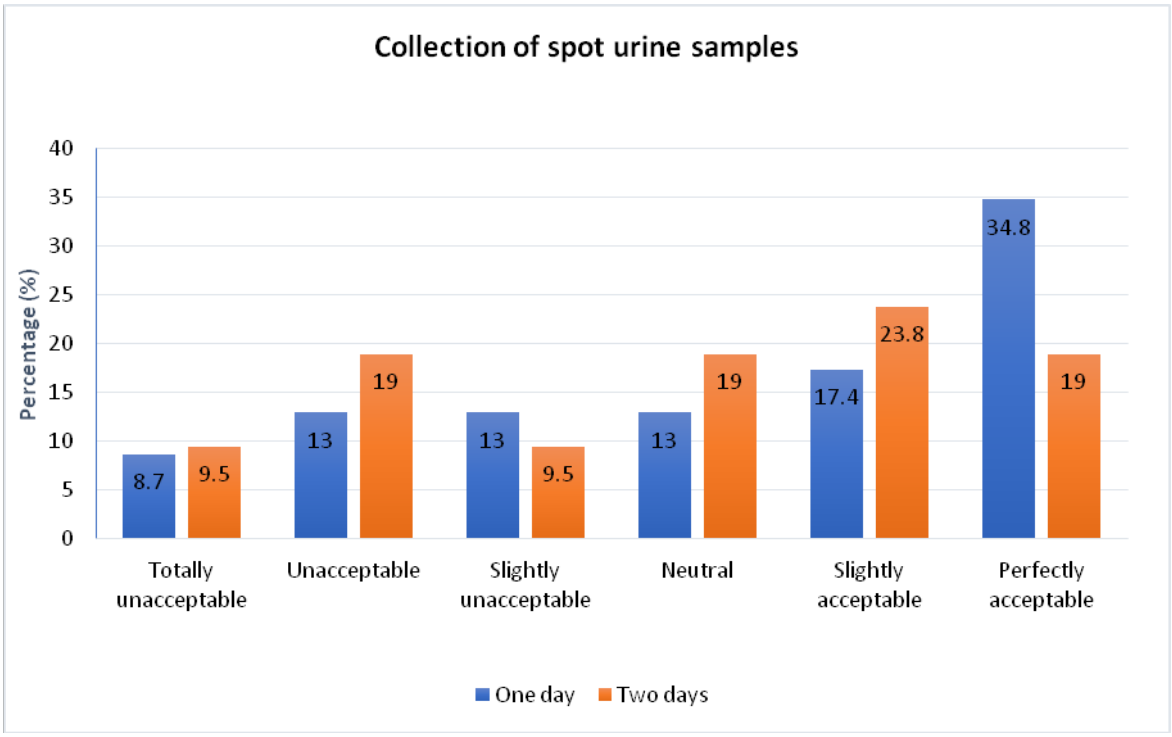

Figure 5.12: What do you feel about having to collect your child's spot urine samples?

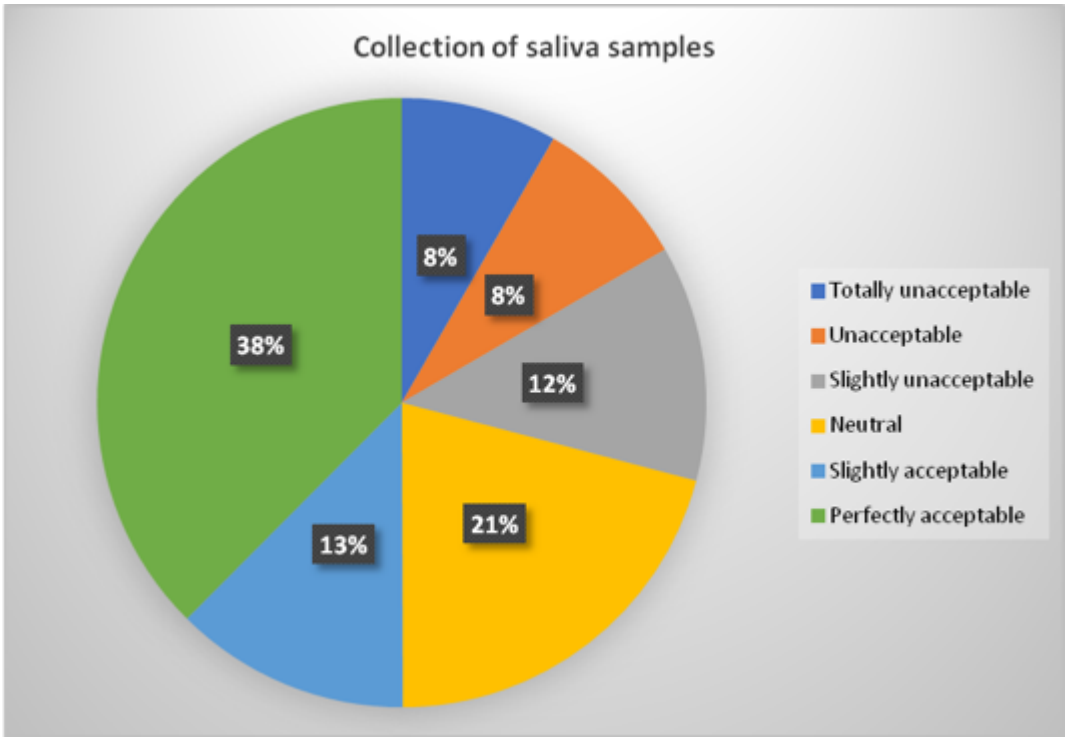

Figure 5.13: what do you feel about the saliva collection from your child?

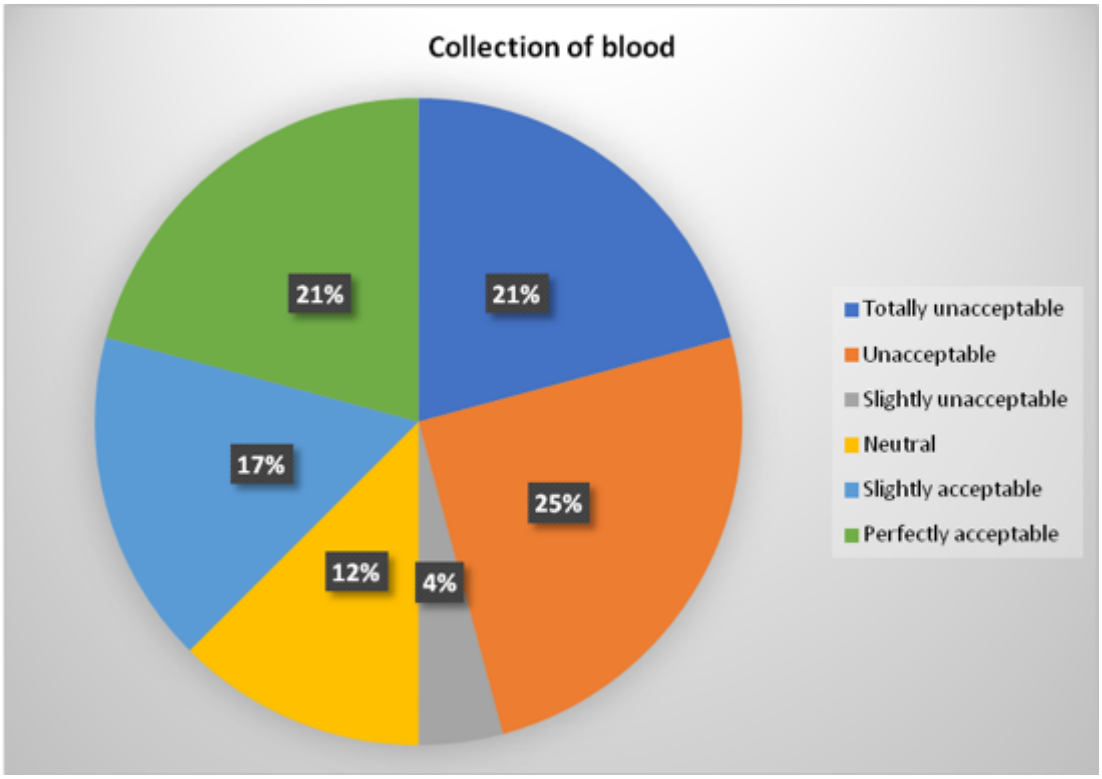

Figure 5.14: How do you feel about the way your child's blood would be taken?

Fifty percent of the participants found collection of their child's hair sample to be perfectly acceptable. The percentage increased to 62% when the same participants were asked if they found collection of nail samples perfectly acceptable, while 38% of respondents felt it was totally unacceptable, unacceptable or slightly unacceptable to collect their child's hair and 28% found their child's nail collection totally unacceptable, unacceptable or slightly unacceptable (Figure 5.15 and 5.16).

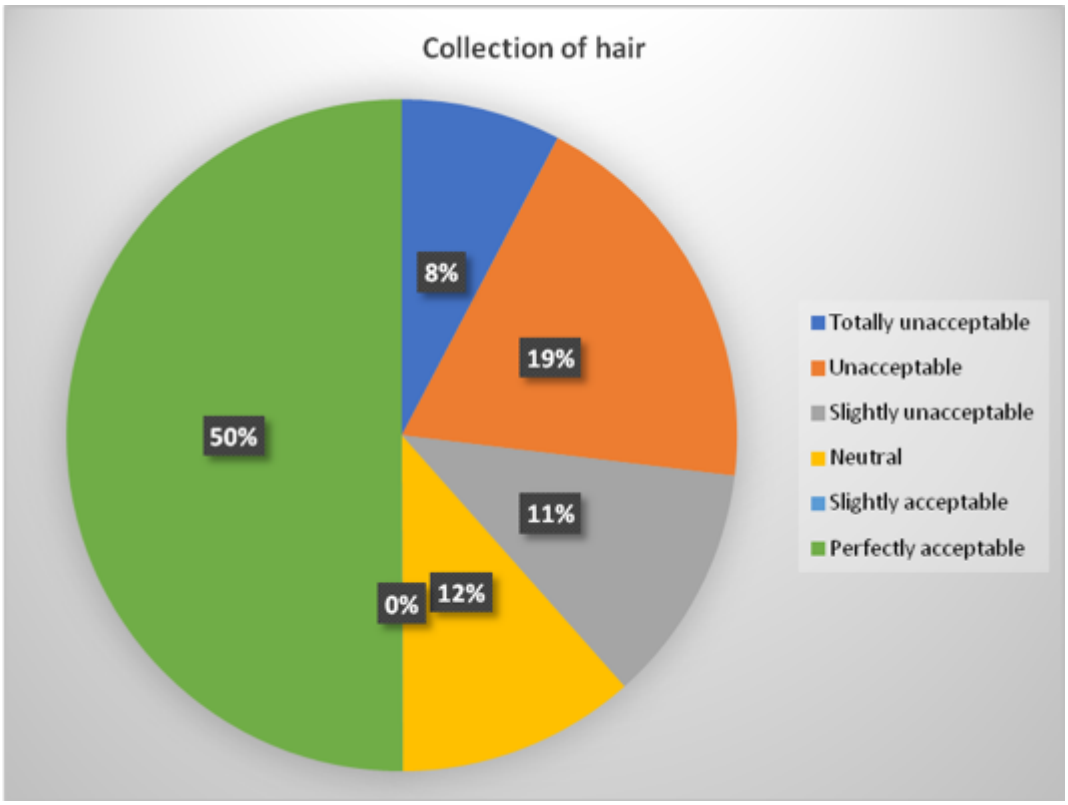

Figure 5.15: What do you think about being asked to collect your child's hair?

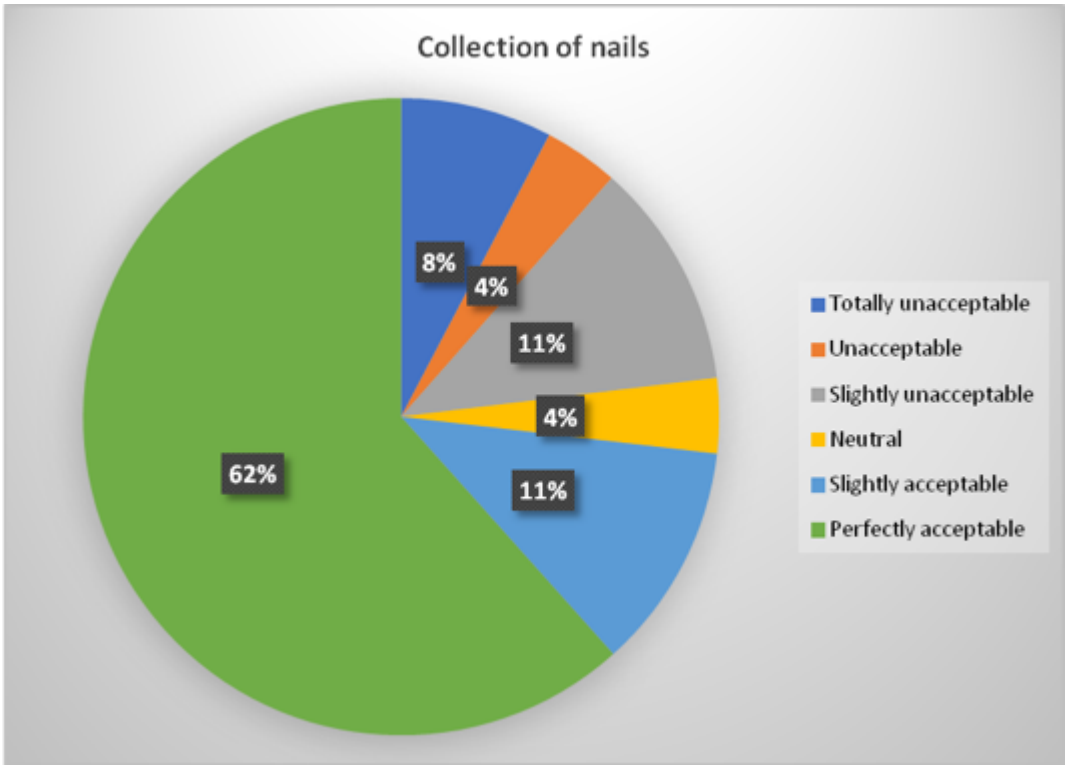

Figure 5.16: What do you think about being asked to collect your child's nails?

B) Perceived ease of collection of the biological marker

- **Adults**

The results from Table 5.5 show the means for how participants would find collecting the biomarkers when asked to provide them. Most of the volunteers found hair collection to be hard (Mean, 4.75) followed by urine and spot urine (Mean, 5.87 and 6.04 respectively) with similar means. However, respondents would find spot urine more-easy to collect compared to a 24-h urine sample. Conversely, participants felt that nail sample collection would be easier (Mean, 8.26) compared to blood and saliva (Mean, 7.13 and 7.15 respectively). There was no difference in how participants felt the way blood and saliva would be collected. Most of the participants would find the collection of the nail, blood and saliva very easy (Mode, 10) followed by 24-h and spot urine samples (Mode, 8). However, most of the participants would find hair collection very difficult (Mode, 1).

Overall, considering the median values from Table 5.5, nails would be very easy to collect while hair would be very hard to collect. However, participants felt indifferent about the collection of 24-h urine and spot urine samples.

Table 5.5: Now that you know what you would have to do to collect …….. Can you answer on a scale of 1-10 by selecting a number how you would find collection of ……. 1 means very hard and 10 means very easy.

Biomarker	N	Mean	Median	Mode
24-hour urine	104	5.87	6.00	8
Spot urine	104	6.04	6.00	8
Saliva	105	7.15	8.00	10
Blood	104	7.13	8.00	10
Nail	103	8.26	10.00	10
Hair	102	4.75	4.00	1

- **Children**

Table 5.6: Now that you know what you would have to do to collect your child's ……..
Can you answer on a scale of 1-10 by selecting a number how you would find collection of ……. 1 means very hard and 10 means very easy.

Biomarker	N	Mean	Median	Mode
24-hour urine	24	4.08	3.00	1[a]
Spot urine	24	4.63	5.00	1
Saliva	24	5.50	6.00	3
Blood	24	4.00	3.00	1
Nail	24	8.50	9.50	10
Hair	24	7.08	8.50	10

a. Multiple modes exist

Most of the participants who completed the survey on behalf of their child would find nails (Mode, 10) and hair (Mode 10) very easy to collect. However, the values of the mean and median revealed that participants would find nails (Mean, 8.50; median, 9.5) very easy to collect compared to hair (mean 7.08; median, 8.50). In contrast, most of the participants felt

collection of 24-h urine (Mode, 1), spot urine (1) and blood (Mode, 1) would be very hard. However, the mean values in Table 5.7 reveal that they felt indifferent about collection of blood samples, 24-h and spot urine samples (Mean, 4.00, 4.08 and 4.63 respectively) from their child. This is also the same for saliva collection (Mean, 5.50), although, the participants would prefer saliva compared to the other contemporary markers (blood, 24-h and spot urine samples).

C) Behavioural interest to use the biomarkers

- **Adults**

According to Figure 5.17, participants were more likely to provide nail samples in the future (85.9%) followed by saliva, blood, spot urine and 24-h urine, with percentages 81.0%, 74.2%, 70.2% and 62.0%, respectively. However, 62% of participants were unlikely to provide hair samples in the future. The result from Table 5.7 also confirms the attitude of participants towards the collection of hair samples, as it was mostly selected by the participants as the least preferred among the biological markers (Mode, 1) and for nails as the most preferred among the biological markers (Mode, 6). Considering that many of the participants also found 24-h urine to be their least preferred (Mode, 1), it becomes difficult to say which of 24-h urine and hair was the least preferred (Median, 3). However, we can conclude based on the means that 24-h urine is the least preferred among the biological markers (Mean, 3.04).

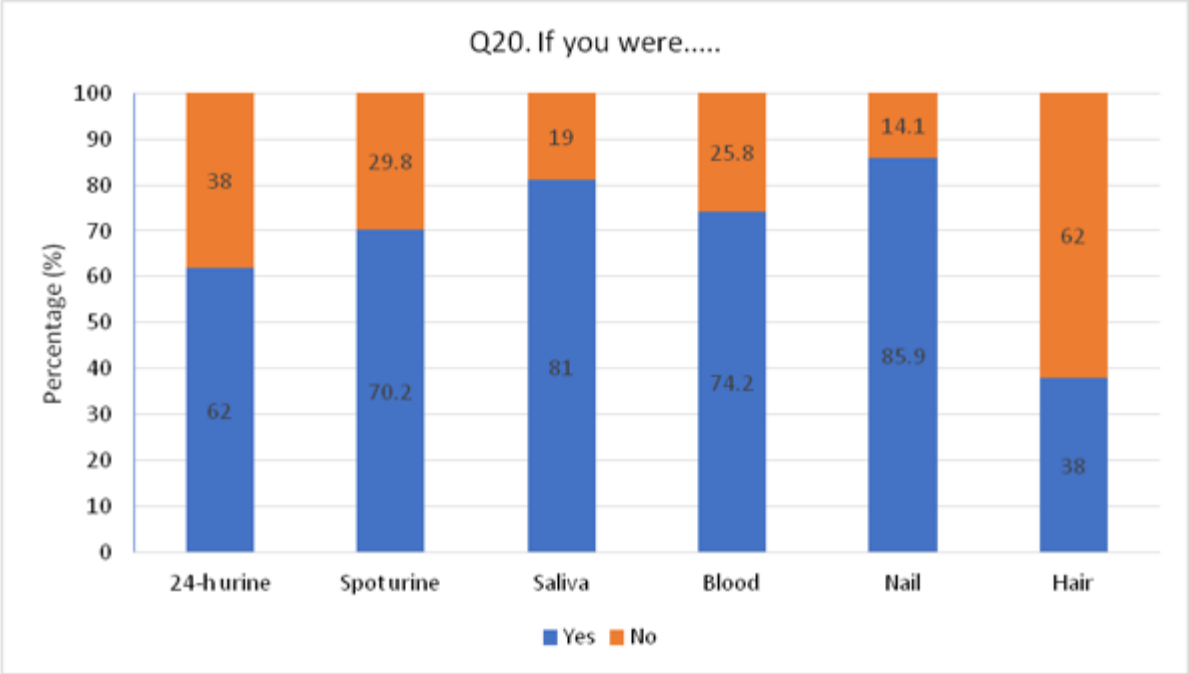

Figure 5.17: If you were asked to provide any of the above types of sample, which would you be willing to provide?

Table 5.7: Please rank in order of 1-6 by selecting a number how you prefer it. 1 means least preferred and 6 means most preferred

Biomarker	N	Mean	Median	Mode
24-h urine	104	3.04	3.00	1
Spot urine	102	3.38	3.00	5
Saliva	102	3.86	4.00	4[a]
Blood	102	3.44	3.00	3
Hair	99	3.11	3.00	1
Nail	102	4.35	5.00	6

a. Multiple modes exist

- **Children**

Figure 5.18 shows that 84% of the participants were most likely to provide nail samples in the future followed by hair (59.1%) and saliva (52.2%) respectively. In contrast, 79.2% revealed that they would not be willing to provide their child's blood when asked. Equally, 69.2% would not be willing to provide their child's 24-h urine samples. The percentage dropped to 65.2% when asked if they would be able to provide spot urine samples. Forty-eight percent of respondents would not be willing to provide their child's saliva while 52.2%

would be able to provide it when asked. The results from Table 5.8 show that nails are the most preferred biomarker (Mode, 6), followed by hair (Mode, 5), while blood, spot urine, and 24-h urine were the least preferred. However, considering the mean values of how participants preferred the biological markers, blood was least preferred (Mean, 2.05), followed by 24-h urine (Mean, 2.78) and spot urine (Mean, 3.23) respectively.

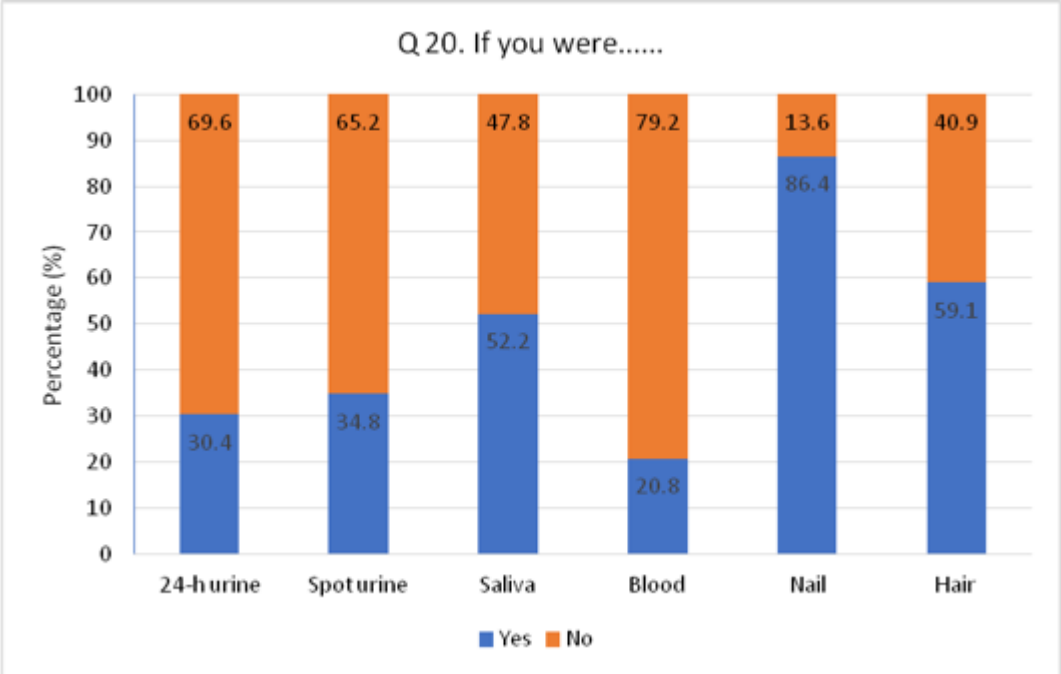

Figure 5.18: If you were asked to provide any of the above types of sample, which would you be willing to provide?

Table 5.8: Please rank in order of 1-6 by selecting a number how you prefer it. 1 means least preferred and 6 means most preferred

Biomarker	N	Mean	Median	Mode
24-h urine	23	2.78	2.00	1[a]
Spot urine	22	3.23	3.00	1
Saliva	22	3.18	3.50	4
Blood	22	2.05	1.00	1
Hair	22	4.04	5.00	5
Nail	23	4.95	6.00	6

a. Multiple modes exist

5.4.1.4 Comparison between adults and children

Figures 5.19 and 5.20 show the level of acceptability in adults and children respectively, i.e. how participants would find utilisation of the biomarkers for monitoring exposure to fluoride. Among adult participants, they would find blood and nail more acceptable compared to the other biomarkers whereas, among children, hair would be more acceptable. Figure 5.21 show the comparism between acceptability in adults and children.

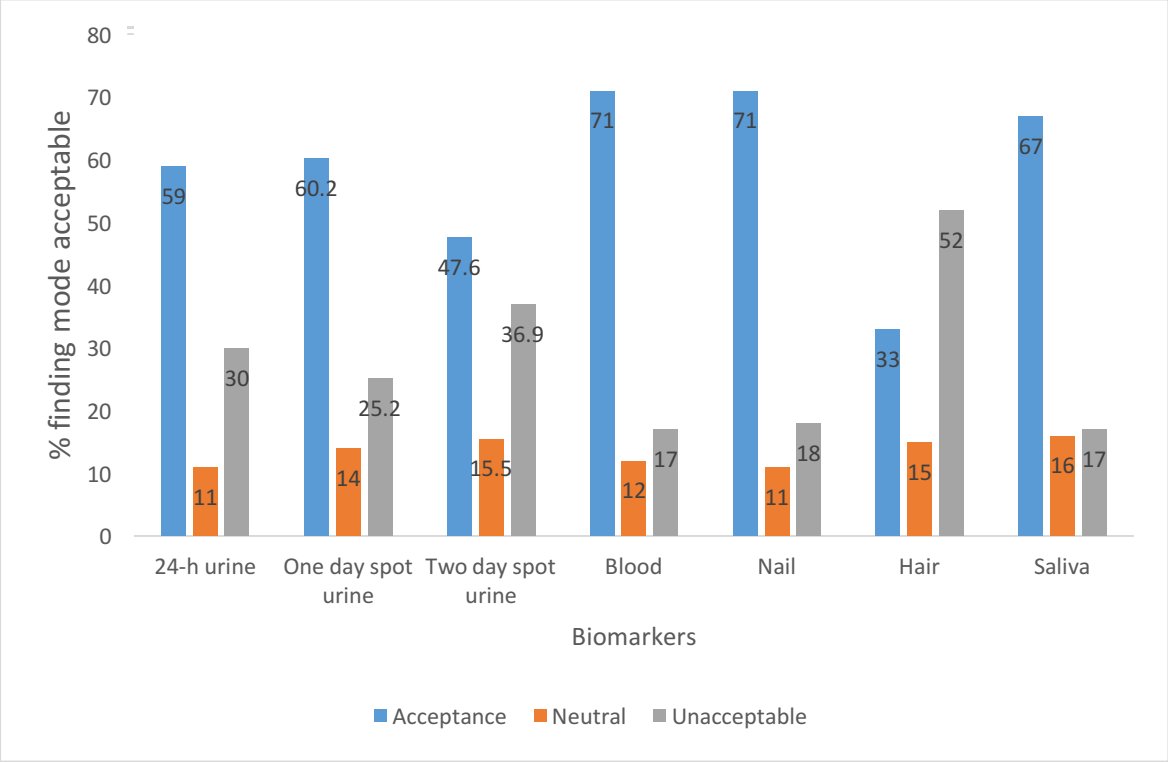

Figure 5.19: Acceptability of the collection of biomarkers of fluoride exposure in adults

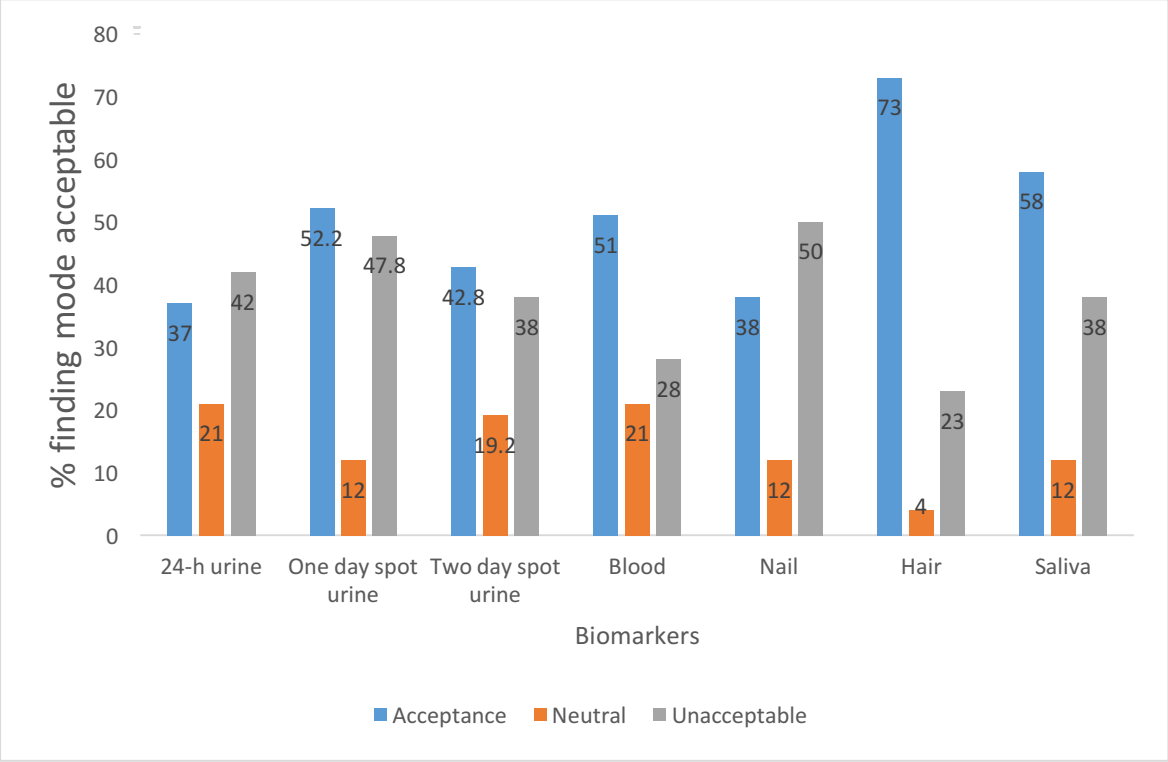

Figure 5.20: Acceptability of the collection of biomarkers of fluoride exposure in children

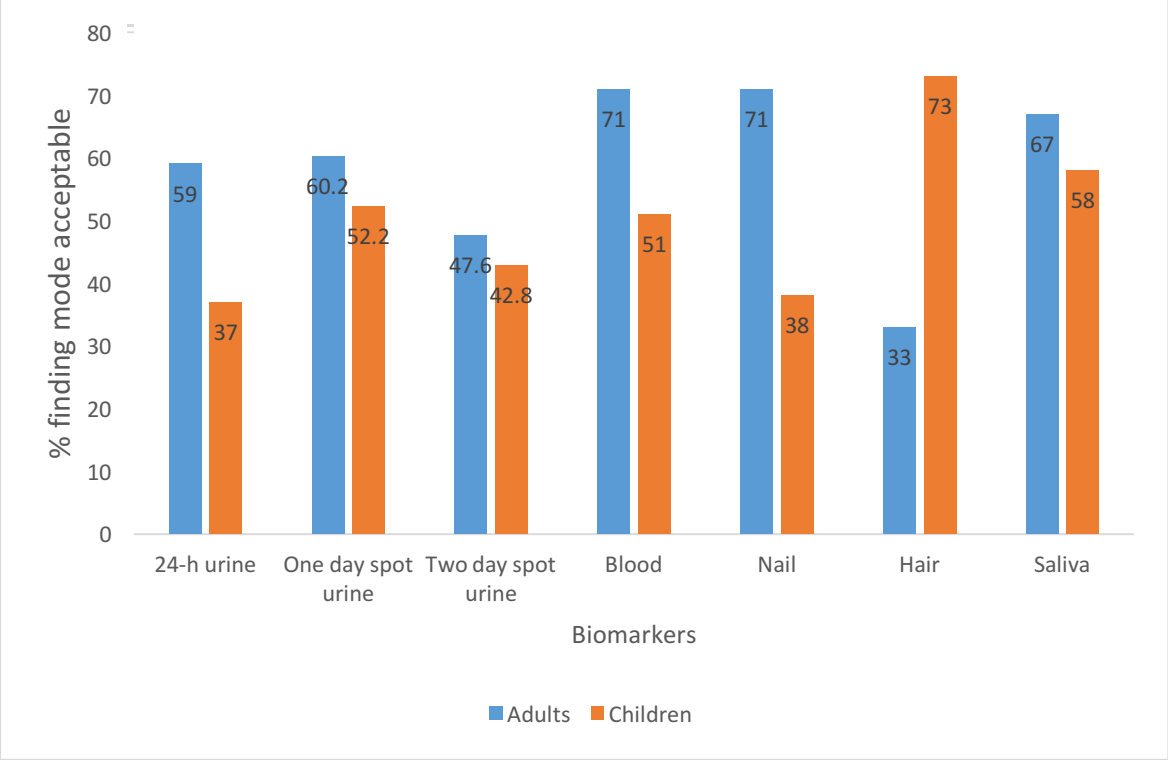

Figure 5.21: Percentage of subjects who found the collection of biomarkers of fluoride exposure acceptable

5.4.1.5 Some socio-demographic factors that influence acceptability

Tables 5.9-5.12 show the association between some socio-demographic factors including gender, ethnicity, profession, and religion respectively on people's perception of the utilization of the different biomarkers for monitoring exposure to fluoride.

Table 5.9 Chi-square test of gender and people's perception on biomarkers

Biomarkers	N	Chi-Square (χ^2)	df	p-value (Sig. 2-sided)
24-h urine	103	5.569	2	0.062
One-day spot urine	100	1.530	2	0.465
Two-day spot urine	100	2.564	2	0.277
Saliva	102	0.288	2	0.866
Blood	100	0.073	2	0.964
Nails	102	0.130	2	0.937
Hair	67	3.060	2	0.217

df stands for degree of freedom

Table 5.10 Chi-square test of ethnicity and people's perception on biomarkers

Biomarkers	N	Chi-Square (χ^2)	df	p-value (Sig. 2-sided)
24-h urine	101	14.230	18	0.714
One-day spot urine	98	20.799	18	0.290
Two-day spot urine	98	20.305	18	0.316
Saliva	100	18.730	18	0.409
Blood	98	12.286	18	0.832
Nails	100	43.770	18	0.001
Hair	65	18.685	18	0.411

df stands for degree of freedom

Table 5.11 Chi-square test of profession and people's perception on biomarkers

Biomarkers	N	Chi-Square (χ^2)	df	p-value (Sig. 2-sided)
24-h urine	70	15.179	10	0.126
One-day spot urine	68	9.103	10	0.522
Two-day spot urine	70	13.840	10	0.180
Saliva	70	7.703	10	0.658
Blood	67	7.315	10	0.695
Nails	70	4.058	5	0.541
Hair	44	7.633	10	0.665

df stands for degree of freedom

Table 5.12 Chi-square test of religion and people's perception on biomarkers

Biomarkers	N	Chi-Square (χ^2)	df	p-value (Sig. 2-sided)
24-h urine	103	5.906	4	0.206
One-day spot urine	100	2.512	4	0.642
Two-day spot urine	100	4.552	4	0.336
Saliva	102	1.241	4	0.871
Blood	100	4.020	4	0.403
Nails	102	2.584	4	0.630
Hair	67	4.713	4	0.318

df stands for degree of freedom

5.4.2 Quantitative study

5.4.2.1 Response rate

A total of 15 (14%) participants accepted to take part in the quantitative study out of the 104 participants who completed the online survey for the qualitative study. However, 3 participants who accepted to take part dropped out from the study as they were away from

the University when the sample collection commenced. Therefore, the response rate for the quantitative study was 12% for the adult age group. Twelve participants provided one or more of the biomarkers as revealed in Figure 5.22, which shows the number of biomarker samples provided by the participants.

Among the child age group, none of the participants in the qualitative study accepted to take part in the quantitative study.

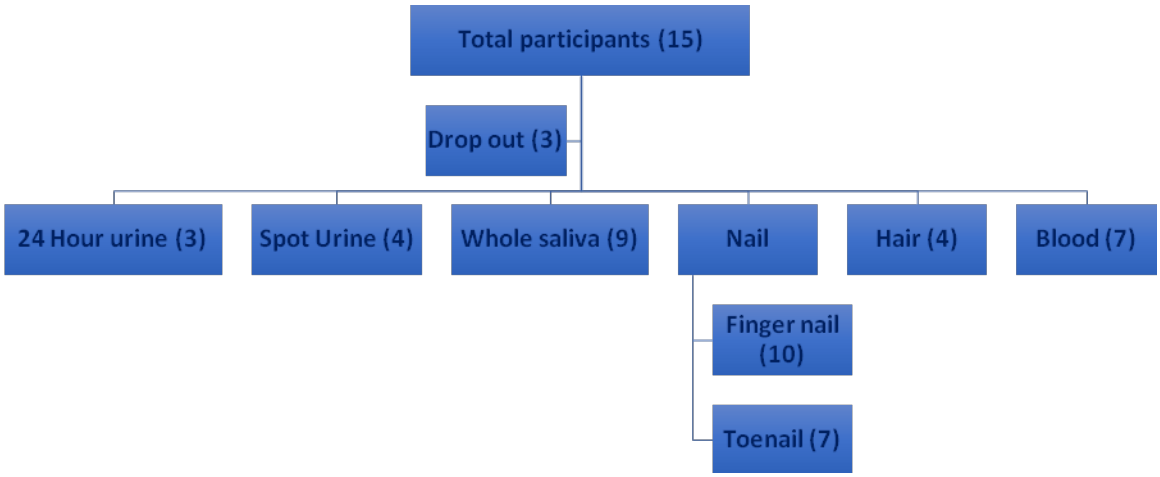

Figure 5.22: Summary of participants involved in the quantitative study

5.4.2.2 Anthropometric characteristics and tap water fluoride of participants

Recruitment for this study was from the subsample of the qualitative study from the adult participants. Data collection was conducted alongside recruitment since only participants who were happy to provide any or all the biomarkers took part in the study. A total of 15 participants completed the response form to take part in the study, out of which three dropped out due to unavailability to provide any of the samples selected. Thus, 12 participants completed the study. In total, 83.8% (n=10) of the 12 participants who volunteered to participate in the fluoride study and provided one or more biomarker samples were females (Table 5.13). The means (SD) for age, weight, height, and BMI of the participants were 40.5 (12.6) years, 68.0 (11.7) kg, 166.0 (6.1) cm, and 24.9 (5.5) kg/m^3

respectively. In total, 12 tap water samples were collected from the participants. The mean fluoride concentration (SD) was 0.233 (0.325) mg/l.

Table 5.13: Gender of participants

Sex	Frequency	Percentage %
Male	2	16.7
Female	10	83.3
Total	12	100

5.4.2.3 Results of data obtained from biological marker samples (adults)

Table 5.16 shows the mean fluoride concentration of the biological markers provided by the adult participants. Mean corrected volume of urine and the urinary flow rate were 969.6 ml/24-h and 40.4 ml/h respectively. Mean (SD) urinary fluoride excretion was 1.524 (0.240) mg/d and 0.020 (0.007) mg/kg bw/d by body weight of participants (n=2). Mean (SD) of the F concentrations in plasma (n=7), saliva (n=9), spot urine (n=4), toenail (n=7) and fingernails (n=8) samples were 0.025 (0.028) µg/ml, 0.172 (0.185) µg/ml, 1.267 (0.592) µg/ml, 0.513 (0.102) and 0.610 (0.341) µg/g, respectively. The results show that fingernail fluoride was numerically higher than that of toenails. However, no statistical data is available to confirm the significance of this difference. This is due to the insufficient number of samples for a statistical analysis to be conducted.

5.4.2.4 Tooth brushing habits and fluoride intake from toothpaste

Considering the oral health habits of the participants (Table 5.14), the study showed that a total of 80% of the participants brushed their teeth twice per day while 20% brushed once a day. Most of the fluoride in the toothpaste used by the participants was a mixture of sodium monofluorophosphate and sodium fluoride, while 25% and 8.3% of the remaining participants used toothpaste containing only sodium fluoride or sodium monofluorophosphate respectively. In total, 90% of the participants used toothpaste that contained 1450 µg/g fluoride while 10% used toothpaste containing 1000 µg/g fluoride. All those who participated in this study used an adult toothbrush and none of them used fluoride varnish or gels.

Fluoride intake was estimated for the adult participants as presented in Table 5.15. Mean (SD) of fluoride ingestion per day was 0.715 (0.240) mg and 0.010 (0.004) mg/kg body weight. The mean (SD) weight of toothpaste dispensed on the toothbrush was 0.70 (0.16) g.

Table 5.14: Tooth brushing habits of participants (Adults)

	Percentage %
No of brushings/day	
- Once	20
- Twice	80
Form of fluoride in toothpaste used	
- Sodium fluoride (NaF)	25
- Sodium monofluorophosphate (SMFP)	8.3
- SMFP + NaF	66.7
Fluoride concentration of toothpaste used	
- 1000 µg/g	10
- 1450 µg/g	90

All participants used adult size toothbrush

Table 5.15: Mean (SD) of fluoride intake from toothpaste (N = 10)

	Range	Minimum	Maximum	Mean (SD)
Weight of toothpaste dispensed on toothbrush (g)	0.50	0.25	0.75	0.70 (0.16)
Fluuoride ingestion per day mg	0.595	0.297	0.892	0.715 (0.240)
mg/kgbw/d	0.012	0.004	0.017	0.010 (0.004)

5.5 DISCUSSION

5.5.1 Qualitative study

5.5.1.1 Recruitment

The sample size (220 made up of 110 adults and 110 children) for this study was derived from the power calculation described in Chapter 4 and this was considered large enough to enhance external validity of the finding. A total of 110 was calculated to be shared among the three socio-economic bands: band 1, 2 and 3 for most deprived, next most deprived and most affluent council wards respectively. The present study shows that no school from the deprived areas was willing to participate in the research study, whereas one school opted to take part in the next most deprived area and two schools from the most affluent areas. Similar results were also obtained when the questionnaires were dispatched to the school located in the next most deprived area (none completed) and those located in the most affluent area (5 were completed). These findings might be associated with the level of education of people living in the affluent areas, as they would be more likely to be aware of the need for such research compared to people living in deprived areas. Interestingly, when adults were told to complete the survey for themselves, there was a 99% response rate but a sharp drop to 23% when they were asked to complete the same survey on behalf of their children. The reason for this discrepancy is not known in the present study. Among the adults who participated in the survey, 95% were from Teesside University and Newcastle University, whereas 5% was completed in the primary school by parents. The level of education might be the reason for the numbers and the awareness about research since it was conducted in the University setting. Most of the head teachers informally reported that the school is busy with different activities and will not be able to fit the study into their programme. A school reported that they are working with so many research studies from the universities and they have decided to limit their contribution otherwise the parents will consider it over load. Others would not accept to participate due to parents not responding to such questionnaires in the past. A few school declined without any reason stated by the head teacher. Support for research from primary schools is increasingly becoming difficult considering their response (Omid 2012). In the present study, there was no difference in their response when the incentive was provided for the school as well as for those who completed the survey.

5.5.1.2 Acceptability of biological markers

The utilization of biomarkers is increasingly important in monitoring exposure to fluoride due to the incidence of dental caries in some areas with low fluoride in drinking water, use of dentifrices, halo effects, consumption of beverages, fluoridation of water, dental fluorosis and other health effects of excessive fluoride exposure. Studies have reported the use of biomarkers for short-term exposures as well as long-term. However, acceptability for the use of these biomarkers of fluoride remains un-investigated. People's perception of the biomarker that will be used in a population, particularly when investigating the efficacy of water fluoridation programme, should be considered when designing and implementing such procedures. Fisher *et al*. (2006) showed that if an intervention is acceptable, patients are more likely to adhere to the treatment recommendation and to benefit from the improved clinical outcome.

The definition of acceptability in the healthcare literature varies considerably, whether social acceptability (Becker *et al*., 2007; Sidani *et al*., 2009) or treatment acceptability (Dillip *et al*., 2012), but a recent overview of acceptability by Sekhon `*et al*. (2017) reported that acceptability can be considered from an individual perspective but may also reflect a more collectively shared judgement about the nature of an intervention. There is currently no literature on acceptability of the use of biomarkers of fluoride exposure but a study conducted among adults in India showed that toenails was preferable to the participants than blood or urine (Sankhala *et al*., 2014). In the present study, Figure 5.21 below shows that nails and blood were the preferred biomarkers among the adults. Interestingly, these biomarkers represent different periods of exposure (Whitford, 1994). According to WHO (1994), blood is a contemporary marker due to its ability to reflect exposure over a short period whereas nail is a recent biomarker of exposure due to its ability to reflect exposure over a longer period. Therefore, in this regard, we might be able to conclude, based on people's perception, that blood is the most acceptable contemporary marker of fluoride exposure whereas nail is the most acceptable recent biomarker of fluoride exposure.

In the present study, we further considered other factors that influence human behavior including perceived ease of use and behavioral intentional use as well as actual use according to Abdullah *et al*. (2016). Most of the participants would find the collection of nails and blood easy as the mode value 10 means very easy to collect for both. However, based on the respective averages of the means when participants were asked how easy the collection of

the samples was based on the short description of the procedure in the questionnaire, the participants would find the collection of nails easy compared to blood. On their behavioral intentional use, similar to the perceived ease of use, nails (85%) and blood (74%) were still the most highly preferred among the biomarkers. Participants would still have intention of providing saliva (81%) above blood which might be associated with many factors identified in the questionnaire, including: "I don't like blood test", "Personally not a fan of needles", "too much experience of procedure hurting excessively", "always makes me feel queasy/faint", "my vein don't always play nice and it often takes some time", “concern about my DNA”. A study conducted in the Kintampo district of Ghana involving 12 focus group discussions and 8 in-depth interviews showed that participants have different views when asked to provide blood in hospital and in research settings following the fact that the use of their blood in hospital was mainly for the diagnosis of diseases but when asked in a research center they doubt what the blood is used for (Boahen *et al*., 2013). This is quite interesting due to the difference in cultural setting and the different reason why they wouldn’t provide blood even though they prefer its utilization. Some studies also reported misconceptions about the use of blood samples during trial studies can have a negative effect on the conduct of the trial similar to the present study (Newton *et al*., 2009; Boahen *et al*., 2013). A study also found apprehension about the blood taken and that participants expressed their concern about how blood samples were used with a few suspicious that researchers sold blood samples (Chatio *et al*., 2016).

The present study is the first to report the acceptability of nails for research purposes and this is very useful because of the non-invasiveness of the collection of the sample. As a biomarker of exposure to fluoride, it is recently being explored as a biomarker of acute, chronic and sub-chronic exposure to fluoride due to the possibility of storage of fluoride over a long period of time without degradation (Czarnowski and Krechniak, 1990; Whitford *et al*., 1999; Correa Rodrigues *et al*., 2004; Buzalaf *et al*., 2006). The nail might, therefore, be a useful biomarker of exposure when monitoring future fluoridation programmes since it is also not affected by physiology such as pH, glomerular filtration etc. that influence urinary fluoride excretion that is recommended by WHO (WHO, 2014).

Surprisingly, when the perception of parents regarding the use of their child's tissues was asked, their choice of biomarker was different. They would prefer hair compared to the other biomarkers and we might associate this with the factors described above regarding blood collection as well as the invasiveness of the sampling procedure following from the fact

from Table 5.6 where most parents indicated that blood collection from their children would be very hard and hair collection would be very easy. A further investigation on the behavioural intentional use showed that blood was the least preferred for monitoring exposure to fluoride in children whereas nail was the most preferred as well hair. This might still be associated with the collection procedure which is non-invasive and no pain for their children. Some parents wrote comments on the online survey: "I would not have my child's blood drawn unless it was for their own medical purpose and absolutely necessary", "this procedure hurts and has never been performed on my child, therefore, I would worry about this sample". The comments above, summarizing why the parents would prefer blood collection for themselves but not for their children, might be experience of the parents in providing blood. The other comment can be linked to what was revealed by Boahen *et al*. (2013) on the different views concerning provision of blood for research and medical purposes. Therefore, we might infer that nail would be acceptable among adults and children for monitoring exposure to fluoride and this information could be useful in an epidemiological study. However, if the blood is intended for medical screening, it might also find acceptance among children and adults.

Figure 5.19 shows the number of adult participants who provided samples, one of the characteristics of acceptability identified by Abdullah *et al*. (2016). Surprisingly, the results also show that nails (fingernail and toenail), blood as well as saliva were provided the most, whereas urine (24-h and spot urine) and hair were provided least. We might, therefore, conclude that nail is the most acceptable among the biomarkers for monitoring exposure but since nail is a recent biomarker, blood could be utilized as a contemporary biomarker for its ability to detect short-term exposure as well as saliva.

5.5.1.3 Potential factors affecting acceptable biological markers for monitoring exposure

A χ^2 test showed that there were no significant differences in gender and religion on how people perceived the biomarkers among adults (see Table 5/9-12). This shows that the choice of biomarker by the participants is not affected by their socio-demographic characteristics. We only conducted this among the adult group due to the sufficient number of respondents for external validity. People's perception on choice for 24-h urine, spot urine (one-day and two-day), saliva, blood and hair was not influenced by ethnicity, but with nail,

the test identified a strong association between participant's perception (χ^2= 43.770, df=18, p<0.01).

To understand some of the reasons why some of the biomarkers were not accepted by the participants of the study, some parents indicated the reason for their decision. For hair, which was the least acceptable among adults, the respondents held some views including "my hair is too short", "unless it was medically required and an absolute requirement I would not allow this", "difficulty re appearance if it would leave a patch without hair", "I pay good money to have my hair cut and you propose to make a mess of it", "I would be ok if it was clipped from the end of my hair but not from the scalp……", "I have long hair so a section close to the scalp will show…….", "hair takes a long time to grow back……", "I don't keep my hair". Among the above factors mentioned, the creation of a patch was most identified by the respondents and the fear of losing some of their hair which might take longer to grow back was why they find it difficult to accept the use of hair.

Only 59% of the total participants would find collection of 24-h urine acceptable, some of the participants highlighted some factors that might limit the utilization of this sample including: "would prefer to do it on a weekend when not at work", "it would be difficult as I work full-time and would find it inconvenient to take samples whilst at work", "this would tie someone at home for 24 hours - people have lives to lead", "it would be more manageable if collected in smaller bottles throughout the day", "only the hygiene factor is putting me off", "……as a woman it was difficult because we were given a funnel and a bottle – was annoying having to take this to the toilet every time and it was difficult to use", "storage at work, convenience of transporting while on the move", "it is really discomforting", "slight embarrassment if required to use public toilets in that 24 hours, a bit uneasy about storing such a large volume of urine". In summary, work-related issues, inconvenient, size of the container for sample collection, hygiene issues, and volume of sample collected as well as transport are factors that might affect the utilization of urine from participants' point of view. However, participants advised that they might find weekend collection acceptable since they would be off work.

5.5.1.4 Limitations of the study

The present study might have some possible limitations like every other study, which should be considered when interpreting the results.

Study setting: The survey in the present study was conducted at the University of Teesside and Newcastle University where two questionnaires were administered. The first was completed by adults for themselves whereas the second was completed by parents of 4-5 years old children on behalf of their children. We decided to adopt this recruitment method due to difficulties encountered in getting schools to participate in the study, particularly in highly deprived areas. A previous study conducted by Lawrence (2012) among 10-12 years old children living in Gateshead, northeast of England showed that parents/guardians living in highly deprived areas are reluctant to return signed documents back to school. An informal discussion with some school authorities revealed that if the school decided to take part in the studies, there was no likelihood of parents returning the questionnaire back to the school. Table 5.2 from the present study showed none of the 45 questionnaires was returned to the school from the parents after the school in a deprived area opted to participate in the study. Therefore, the representativeness of this present study for the different socioeconomic backgrounds will have been affected.

Sample size: In the present study, the total sample size from the power calculation was 220, representing 110 adults and 110 children. In the adult study, there was 99% completion whereas, in the child survey, only 25% completed the survey showing that the sample size for the child survey is not considered large enough for the external validity of the findings.

Study characteristics: The adult participants for the present study represent the various ages in the population due to their wide distribution across the age groups. However, there is no balance in gender, ethnicity, level of education and religion. Majority of the participants in the present study were female (64%), white (62%), educated to degree level (81%) and Christian (62%). Similar demographic information was also found among parents who completed the child survey.

5.5.1.5 Conclusions

The biomarkers of fluoride are very useful primarily for monitoring deficient and excessive intakes of biologically available fluoride and these have been published in the literature (Rugg-Gunn *et al*., 2011; Pessan and Buzalaf, 2011). However, people's perceptions about their willingness to be able to provide samples are uninvestigated. The present study showed that nail was acceptable for use among adults for monitoring exposure to fluoride as well as blood but some participants were concerned about the provision of blood particularly for research purposes due to the fear of what their sample would be used for. Among children,

blood seems not to be acceptable but hair was their preferred choice probably due to the non-invasiveness of the procedure.

5.5.2 Quantitative study

This section focuses on the discussion of the results of fluoride concentration in the biomarkers provided by adults who opted to take part in the fluoride exposure studies. They completed the demographic form, fluoride exposure questionnaire containing the tooth brushing habits and provided some biomarkers which were analysed for fluoride (see protocol in Chapter 4).

5.5.2.1 Recruitment

The participants for this study were a subset of those adults/parents who completed the online survey. A total of 12 participants took part in the studies and provided the different samples among 24-hour urine, whole saliva, blood, nails (fingernail and toenail) and hair depending on their choice (see Figure 5.20) by themselves, except blood which was collected by a nurse (see sample collection in Chapter 4). More females (83%) took part in the sample provision than males and this is associated with the fact that more females participated in the online survey.

5.5.2.2 Anthropometric characteristics/ Tooth brushing habits

According to the WHO (2004) Body Mass Index (BMI) classification, the participants were in the normal range of BMI (24.9 kg/m^2). It was interesting to find out that most of the adults brushed twice per day using toothpaste containing 1450 µg/g fluoride concentration recommended in the UK (NHS), which is available in the form of sodium monofluorophosphate (SMFP) and sodium fluoride (NaF) combined. However, the effect is small and both forms of fluoride are beneficial. The participants, therefore, have healthy brushing habits.

5.5.2.4 Fluoride concentration in biomarkers

In the present study, the average amount of toothpaste placed on the toothbrush was 0.70 g and the intake of fluoride from this toothpaste was 0.715 (0.240) mg/d and 0.010 (0.004) mg/kgbw/d when adjusted by body weight. There are no reports on adult toothpaste use in England. However, in the present study, the mean weight of toothpaste used by adults was similar to that (0.67 g) reported among 4-6 years old children in England (Zohoori *et al*.,

2012). The intake from toothpaste in adults was low compared to what was reported among 4-6 years old English children (0.029 mg/kgbw/d) (Zohoori *et al*., 2012), 4 years old Brazilian children living in fluoridated (0.031 mg/kgbw/d) and non-fluoridated areas (0.029 mg/kgbw/d) (Zohoori *et al*. 2013). This was in line with previous studies which suggested that younger children often ingest a large portion of the toothpaste dispensed on their tooth brush (Oliviera *et al*., 2007; Martin *et al*., 2008) due to their immature swallowing reflex.

Plasma fluoride concentration in the present study was 1.308 µmol/l on average, which is higher than that reported among 27-56 years old (1.00 µmol/l) (Ekstrand 1978), 27-36 years old (0.70 µmol/l) (Ekstrand *et al*., 1981) and 26-38 years old (0.49-0.65 µmol/l) (Oliveby *et al*., 1989) adults living in Sweden, among 20-35 year olds in the UK (1.04 µmol/l) (Maguire *et al*., 2005), and among 25-35 year olds (0.36-0.55 µmol/l) (Cardoso *et al*., 2005), 27-33 year olds (0.53 µmol/l) (Cardoso *et al*., 2008) and 19-29 year olds (1.11-1.16 µmol/l) (Buzalaf *et al*., 2008) in Brazil. However, the concentration in the present study is lower than the concentration (1.84 µmol/l) reported by Ekstrand (1978) among 10-38 year olds with fluoridated drinking water with fluoride concentration, 9.60 mg/l. The relatively high plasma fluoride concentration found in the present study might, therefore, be associated with their high total daily fluoride intake which was not investigated in this study since participants were fasting prior to collection. The participants were advised not to eat any food, drink and as well not to brush their teeth in the morning of the sample collection. This was to estimate their plasma fluoride concentration under resting conditions. Similarly, the previous values reported above were resting plasma concentrations. The high plasma concentration cannot be associated with the drinking water fluoride concentration of the participants but other sources since the average fluoride concentration from drinking water was low 0.233 (0.325) mg/l, similar to the reported value (0.25mg/l in drinking water) by Ekstrand et al. (1977, 1978) when resting plasma fluoride concentration was 0.54 and 0.50 µmol/l respectively.

Urinary fluoride excretion in the present study was 1.506 mg/d and 0.019 mg/kgbw/d when adjusted by body weight, close to the value (1.642 mg/d) reported among 20-35 year olds in Brazil who were exposed to fluoride from diet, dentifrice and 1.8 mg NaF (fluoridated solution) (Buzalaf *et al*., 2006) but the value is higher than that reported for 20-40 year olds (1.24 mg/d) exposed to 1.82 mg/d dietary fluoride intake (Villa *et al*., 2008). However, a recent study conducted in the Rift valley of Ethiopia showed a very high average urinary

excretion of 5.7 mg/d associated with the high fluoride concentration of the drinking water. Fluoride concentration in a single spot urine sample may vary considerably with the fluoride in 24-h urine sample, surprisingly, in the present study, fluoride concentration in spot urine samples (1.517 mg/l) was similar numerically to the 24-h urinary fluoride concentration but due to the limited number of samples collected in the present study, the relationship between the two was not investigated. An early study conducted by Zipkin *et al*. (1956) showed that fluoride concentration in 3 spot urine sample collected from 9 men (0.9 mg/l, 1 mg/l and 1.1 mg/l) did not vary significantly from the mean fluoride concentration of the 24-h urine sample (0.9 mg/l). Watanabe *et al*. (1994) found, among eight 21 years old females, high correlation coefficients between the concentrations of spot urines (collected at 22:00, 7:00 and 8:30) and 24-hr urine fluoride excretion when collected after meal intakes, and between measured concentrations and specific gravity- or creatinine-corrected concentrations in the spot urines. This shows the usefulness of spot urine collected at specific times after meals as done in the present study and can be used as an indicator of the body burden of fluoride (Zober *et al*. 1977; Kono *et al*. 1987; Watanabe *et al*., 1994).

In the present study, mean fingernail and toenail fluoride concentration was 0.610 µg/g and 0.513 µg/g respectively (Table 5.16). The value for fingernail was close to 0.67 µg/g reported among 63 adult women aged 25-50 years old with TDFI 0.33 mg/kgbw/d (Linhares *et al*., 2016). As reported in previous studies, fingernail fluoride was higher than that of the toenail (Levy *et al*., 2004; Buzalaf *et al*., 2009; Amaral *et al*., 2014), which is due to differences in the growth rate of fingernails and toenails thereby reflecting different periods of exposure. This are discussed in more details in Chapter 8 (studies conducted in Nigeria).

Table 5.16 Results of fluoride concentration (F-con) in biomarkers and drinking water samples (* male participants)

Participants	Drinking water fluoride conc. (mg/l)	Biomarker samples						
		UFE (mg/d)	UFE (mg/kgbw/d)	Spot urine F-con. (µg/g)	Saliva F-con. (µg/g)	Plasma F-con. (µmol/l)	Toenail F-con. (µg/g)	Fingernail F-con. (µg/g)
1	0.072	-	-	-	0.150	1.000	0.542	0.741
2	-	-	-	-	-	0.737	-	-
3	0.093	1.694	0.024	-	0.065	0.579	0.566	0.552
4	-	1.471	0.020	1.297	0.620	1.105	-	1.362
5	-	-	-	1.792	-	-	0.565*	-
6	0.076	-	-	-	0.194	4.479	0.325	-
7	0.057	-	-	1.432	0.039	0.842	-	0.302*
8	0.220	1.354	0.015	1.542	0.025	0.316	0.591	0.527
9	0.130	-	-	-	0.205	-	-	0.409
10	0.036	-	-	-	0.033	-	-	-
11	0.954	-	-	-	0.216	-	0.583	0.662
12	-	-	-	-	-	-	0.416	0.322
Mean	0.233	1.506	0.019	1.517	0.172	1.308	0.513	0.610
SD	0.325	0.173	0.005	0.210	0.185	1.466	0.102	0.341

5.5.2.5 Conclusions

The adults in this study were receiving sufficient amounts of fluoride from toothpaste alone for the prevention of dental caries but the intake from diet was not determined. The present study investigated different biological markers of exposure to fluoride and found they could be used for monitoring fluoride exposure. Plasma fluoride concentration from a non-fasting subject is affected by total daily fluoride intake and recent exposure thereby making the fluoride concentration higher than plasma fluoride concentration collected from fasting subjects. The present study found that spot urine sample collected at specific time can be an indicator of body burden of fluoride due to its similarity with the urinary fluoride excretion. Toenail and fingernail fluoride concentration reflect different periods of exposure due to the differences in their growth rate. This study has demonstrated that the different biomarkers can be used in estimating exposure to fluoride. On the basis of the oral hygiene information, the present data suggested that majority of adults brushed their teeth twice per day with adult toothpaste containing 1450 μg/g and they were receiving tolerable amount of fluoride sufficient to protect them against dental caries.

5.6 RATIONALE OF THE SELECTED ASSESSMENT METHODOLOGY

In the qualitative study, two approaches were adopted for the completing of the questionnaire including completion of a paper questionnaire or a web-based questionnaire. The selection of assessment method by the researcher in the present study was due to the practicality of collecting the data from the targeted participants, from the parents of children in Middlesbrough primary schools and staff of targeted universities and response rate. Both options (paper and web-based) were given to the parents in the UK to choose based on their preference. All participants (parents of 4-5 year olds) from selected primary schools completed a paper questionnaire passed on through the teachers. In contrast, participants in the university completed a web-based questionnaire with similar questions uploaded from the paper questionnaire. The present study shows there was more response from participants from the web-based questionnaire compared to the paper questionnaire and the fact that university staff were more likely to respond to their emails than conventional letters received by post or in their office pigeonholes. The result was similar to the finding of Van Den Berg et al. (2011) among younger participants (mean age 30 years), but other studies have revealed a greater response to a paper questionnaire compared to a web-based questionnaire (Kongsved et al., 2007; Zuidgeest et al., 2011). The differences in the response rate might

be attributed to many factors, including motivation of participants associated with the knowledge and relevance of the disease investigated (Kongsved et al., 2007; Zuidgeest et al., 2011), age (van Den Berg et al., 2011), incentive (Hohwu et al., 2013) etc.

The strength of the present methodology is that the adopted assessment method is practical for a high response rate for the whole population. Furthermore, the researcher could easily follow-up the participants from the different groups (primary school and university). The approach also eliminates bias judgement where the researcher is present during the completion of the questionnaire. The method also has a specific limitation, considering the result of the study was drawn from the combination of web-based and paper questionnaire, it might be difficult to identify any form of bias associated to the type of questionnaire that was completed.

5.7 OVERALL CONCLUSION

On the basis of perceived acceptability of biomarker of exposure to fluoride, the presented study concluded that nail is the most preferred biomarker for monitoring fluoride exposure in adults and children whereas the least preferred biomarker among adults is hair. Interestingly, in the UK people keep hair to enhance their outward beauty. However, among children, parents least preferred blood to be taken from their children which might be because of the invasiveness of the procedure. The quantitative study conducted among the adults also supports this finding, since the most provided sample was fingernail (10 participants) whereas hair and 24-h urine samples were the least provided samples. The present study therefore, concluded that nail is the most acceptable marker for monitoring exposure to fluoride since the quantitative study also confirms its usefulness.

CHAPTER 6 – STUDY TWO: ASSESSMENT OF RELIABILITY OF BIOLOGICAL MARKERS OF EXPOSURE TO FLUORIDE IN NIGERIA-METHODOLOGY

6.1 INTRODUCTION

Early investigations demonstrated that between 0.05-0.07 mg/kgbw/d and an upper limit of 0.1 mg/kgbw/day is optimal for caries prevention, fluorosis avoidance or a combination of both (Burt and Eklund, 2005). Ekstrand (1989) reported that the upper limit of fluoride intake for young children should be around 0.05 mg per 100 kcalories of energy intake. Studies also revealed that the fluoride intake corresponding to the consumption of 1 ppm water fluoride was about 0.05 mg/kgbw/d (McClure, 1943). Excessive ingestion of fluoride leads to the development of dental fluorosis and at very high dose might result in skeletal fluorosis. The fluoride level was shown to be low in most parts of Nigeria, 0.3 ppm or less, but exceeded 1.5 ppm recommended by the World Health Organisation (WHO) in some parts of North Central, Nigeria and up to 6.7 ppm in one community (Akpata *et al*., 2009). Dental fluorosis has been shown to be endemic in this area: 26% of sampled10-19 year olds in Lantang Plateau and 51% of 12-15 year olds in Plateau and Bauchi State. However, it was observed that some children born and raised in these high fluoride areas did not show evidence of dental fluorosis (El-Nadeef and Hokala, 1998; Wongdem *et al*., 2002; Debal *et al*., 2008).

It has been reported that major contributions to fluoride intake include artificially -or naturally fluoridated water, food and drinks prepared with such water, dietary supplements e.g. fluoridated milk; non-dietary fluoride supplements e.g. mouth rinses and gels; and other caries prevention agents such as tablets (Zohoori and Rugg-Gunn, 2000). Fluoridated toothpaste also contributes to the systemic intake of fluoride, especially in children. Estimation of total daily fluoride intake is important when recommendations of fluoride use are being considered for dental caries prevention while minimising the risk of fluorosis. To quantify fluoride intake from the diet, several dietary assessment methods have been used including: market basket (Ophaug *et al*., 1980), 7-day food record (Schamschula *et al*., 1988), 3-day diary (Maguire at al., 2007; Zohoori *et al*., 2006), duplicate method (Franco *et al*., 2005), food frequency questionnaire (Miziara *et al*., 2009) as well as fluoride exposure questionnaires (Martinez-Mier and Soto-Rojas, 2010). Estimation of fluoride in toothpaste is also difficult but a valid and reproducible method has been used in measuring and

analysing the amount of toothpaste dispensed during tooth brushing and of expectorated saliva by some authors (Maguire at al., 2007; Zohoori *et al*., 2006). These methods are usually time-consuming, costly and require a high level of expertise. In addition, the transport of food and drinks from fluoridated to non-fluoridated areas and vice-versa has also made it difficult in estimating fluoride exposure (Maguire and Zohoori, 2013). The increasing movement of processed foods and drinks across water fluoridation boundaries has resulted in a halo effect, making it more difficult to quantify total fluoride intake. Also, the fluoride concentration of water, just by itself, cannot predict the development of fluorosis. Therefore, the use of biomarkers, including blood, bones, teeth, and urine has been helpful. In addition, fluoride concentration in saliva, milk, and sweat might reflect blood fluoride and fluoride concentration in nails (finger and toe) and hair might reflect past blood fluoride concentration and the body burden of fluoride (Rugg-Gunn *et al*., 2011).

Studies have been conducted both in animals and humans to provide evidence with regards to the accuracy of these biomarkers in determining fluoride exposure (Schamschula *et al*., 1985; Czarnowski and Krechniak, 1990; Furlani *et al*., 2001; Buzalaf *et al*., 2004). A study also reported a better correlation between fluoride intake and nail fluoride content compared with urine fluoride excretion (Buzalaf and Whitford, 2011). Urine, fingernails, head hair, saliva, and plaque in the study of Schamschula (1985) also showed consistently increased fluoride concentrations but not proportionate to increasing water fluoride levels. Few other reports have compared some of these biomarkers and the evidence from those studies is inconsistent (Elsair *et al*., 1982; Czarnowski and Krechniak, 1990; Kono, 1997; Susheela *et al*., 2013), as most of these studies did not take into consideration fluoride intake from all sources. Consequently, there is a need for further investigation comparing both the recent and contemporary biomarkers of exposure to fluoride to obtain the most reliable biomarker. Also, for a biomarker of fluoride exposure to be applicable in a large population, it should be easily collectible without objections from the donors. In this regard, the research will also assess the level of acceptability for the collection of these biomarkers among different age groups.

This chapter presents the aims, methods (including results to make choice of region) of the study conducted in Nigeria.

6.2 AIMS AND OBJECTIVES

6.2.1 Aim

The aim of the study was to find the most reliable biological marker (s) of exposure to fluoride among contemporary and recent biomarkers.

6.2.2 Objectives

- Measure total fluoride intake from diet (food and drinks) in two age groups (4-5 and ≥20 years) by Food Frequency Questionnaires (FFQ).
- Measure fluoride intake from toothpaste in two age groups (4-5 and ≥20 years)
- Measure the concentration of fluoride in 24-hour urine, whole saliva, plasma, hair, nails (fingernail and toenail) and blood in children and adults.
- Estimate daily urinary fluoride excretion (mg/d and mg/kgbw/d) and daily fluoride retention (mg/d and mg/kgbw/d)
- Investigate correlations between fluoride concentration in drinking water (µg/l) supply and i) urinary fluoride excretion (mg/kgbw/d), ii) fluoride concentration in hair (µg/g), iii) fluoride concentration in nails (fingernail and toenail) (µg/g), iv) fluoride concentration in saliva (µg/l), v) fluoride concentration in blood (µg/l).
- Investigate correlations between total fluoride intake (mg/d and mg/kgbw/d) and i) urinary fluoride excretion (mg/kgbw/d), ii) fluoride concentration in hair (µg/g), iii) fluoride concentration in nails (fingernail and toenail) (µg/g), iv) fluoride concentration in saliva (µg/l), v) fluoride concentration in blood (µg/l).
- Investigate correlations between daily urinary fluoride excretion (mg/kgbw/d) and i) fluoride concentration in saliva (µg/l), ii) fluoride concentration in blood (µg/l), iii) fluoride concentration in hair (µg/g) and iv) fluoride concentration in nails (fingernail and toenail) (µg/g).
- Investigate the effect of age and fluoride area on i) urinary fluoride excretion ii) fluoride concentration in saliva iii) fluoride concentration in plasma iv) fluoride concentration in fingernails v) fluoride concentration in toenails vi) fluoride concentration in hair.

6.3 PRELIMINARY STUDY

6.3.1 Aim

The aim of this study was to investigate fluoride concentration in drinking water in some selected LGAs in Plateau state, Nigeria.

6.3.2 Study location

Plateau state was selected based on the previously reported wide range of drinking water fluoride concentration of 0.12-10.30 mg/l (Akpata *et al*., 2009; Dibal *et al*., 2012) as well as the reported dental caries and dental fluorosis prevalence in the area. In order to identify high and low fluoride areas for the present study, the researcher (Idowu) visited the region in February 2015 and collected water samples from various sources in randomly selected LGAs in Plateau state. Plateau state has three senatorial districts; Plateau south, Plateau north, and Plateau central. According to previous studies, Plateau-central and Plateau north senatorial district are located on Jos Plateau with rock formations showing low ground water fluoride, while the geology of Plateau south senatorial district shows some areas with high fluoride in ground water. In each senatorial district, three LGAs were randomly selected except for Plateau south where Langtang north was purposely selected due to its high concentration of fluoride in drinking water as reported from previous literature (Dibal *et al*., 2012). In total, 7 LGAs (40% of all LGAs in Plateau state) were visited, including: Bokkos, Mangu, Pankshin from Plateau central senatorial district and Jos east, Jos south, and Barkin Ladi from Plateau north senatorial district representing the low fluoride area while Langtang north from Plateau south senatorial district was selected for the high fluoride area.

6.3.3 Sample collection

About 100 ml of water was collected in polyethene bottles from all drinking water sources at the headquarters (town where the Local Government council is located) of each selected LGA, as well as from a locality which was randomly selected from the LGA. The bottles were rinsed 3 times with the source water before sample collection. If only one central water supply unit was present in a town, then sampling was made from that source. For places with rivers, ponds, wells or boreholes, one of each type of water was randomly selected. The drinking water sources selected had been in use for at least 5 years. In total, 15 water samples were transported to the United Kingdom for fluoride analysis in the fluoride laboratory, School of Health and Social Care, Teesside University.

6.3.4 Sample analysis

The fluoride concentration of water samples was measured by the direct method in triplicate, at room temperature using a fluoride ion selective electrode and meter after adding TISAB III (Martinez-Mier, 2011). Prior to these measurements, the electrode was calibrated using a series of standards (0.01, 0.1, 1.0, 10, 100 ppm F) prepared by adding TISAB III in a proportion of 1:10 (v/v). The concentration of fluoride standards was chosen to ensure that they covered the range of expected sample concentrations (Martinez-Mier *et al*., 2011). The researcher was trained on how to measure fluoride concentration in various samples (food and drink samples) using an F-ISE by the direct and indirect method at Teesside University prior to the commencement of the analyses.

6.3.5 Results

Mean values of F concentration in drinking water were calculated for each ward in the LGAs as shown in Table 6.1. The LGA with the lowest mean water fluoride concentration was selected for low fluoride; Bokkos LGA had the lowest fluoride concentrations of 0.011 mg/l and 0.046 mg/l in the borehole and well respectively. The three areas selected from Langtang north proved to have the highest fluoride concentrations, ranging from 2.138 to 4.031 mg/l in boreholes.

Table 6.1: Mean fluoride concentration of selected water sources in Plateau state

Study location	LGA	No of samples	Sampling point	Mean (SD) drinking water F conc. (mg/l)
Kufang	Jos south	3	Borehole	0.257 (0.021)
Kufang	Jos South	2	Tap	0.527
Shen	Jos South	3	Borehole	0.696 (0.013)
Shen	Jos South	4	Tap	0.211 (0.012)
Bokkos HQ	Bokkos	5	Borehole	0.011 (0.017)
Bokkos HQ	Bokkos	4	Well	0.046 (0.011)
Mangu HQ	Mangu	3	Borehole	0.036 (0.016)
Dorong	Jos East	3	Stream	0.280 (0.022)
Zakupang	Jos East	2	Well	0.054
Bajin Kogi	Barkin Ladi	4	Well	0.256 (0.023)
Bak	Barkin Ladi	3	Borehole	0.111 (0.015)
Pyache	Langtang north	3	Stream	0.441 (0.014)
Kuyin	Langtang north	2*	Borehole	3.915
Bapkwai	Langtang north	2*	Borehole	2.138
Batkilang	Langtang north	2*	Borehole	4.031

**** Samples were collected from the same source***

6.3.6 Conclusion

Based on the results of this preliminary study, the following two areas were selected for the main study: Bokkos LGA where people receive low fluoride (<0.05 mg/l) in their drinking water and Langtang LGA where the drinking water contains high fluoride (2.1-4.0 mg/l).

6.4 Main study – Material and Methods

6.4.1 Study location

The main study was carried out in Plateau state which is situated in the center of Nigeria between latitude 80°24'N and longitude 80°32' and 100°38' east, consisting of 17 Local Government Areas (LGAs) with a population of 3,383,027 million (1,694,949 males and 1,688,078 females). Plateau state is made up of Precambrian basement complex rocks and younger granite of volcanic origin and it is divided by a mountainous area, Jos plateau, granite up thrust with varying elevation from 900 to 1700 m in the Shere Hills, east of Jos. Agriculture is the predominant occupation of the people of the state, although a significant proportion of the people engage in mining (See Figure 6.1A showing children after the days' work on the farm). Fluoride concentration in the basement aquifers and younger granite aquifers is between 0.003 to 10.30 mg/L and 0.00 to 0.89 mg/L respectively, which is considered low in some areas and higher in other areas compared to the value of between 0.5 mg/L and 1.5 mg/L recommended by the World Health Organization (WHO). It is estimated that 54% of Nigeria's population live in poverty with 70.8% living below the poverty line of less than US$1/day, with more poverty in Northern Nigeria where Plateau state is located and more in the rural parts.

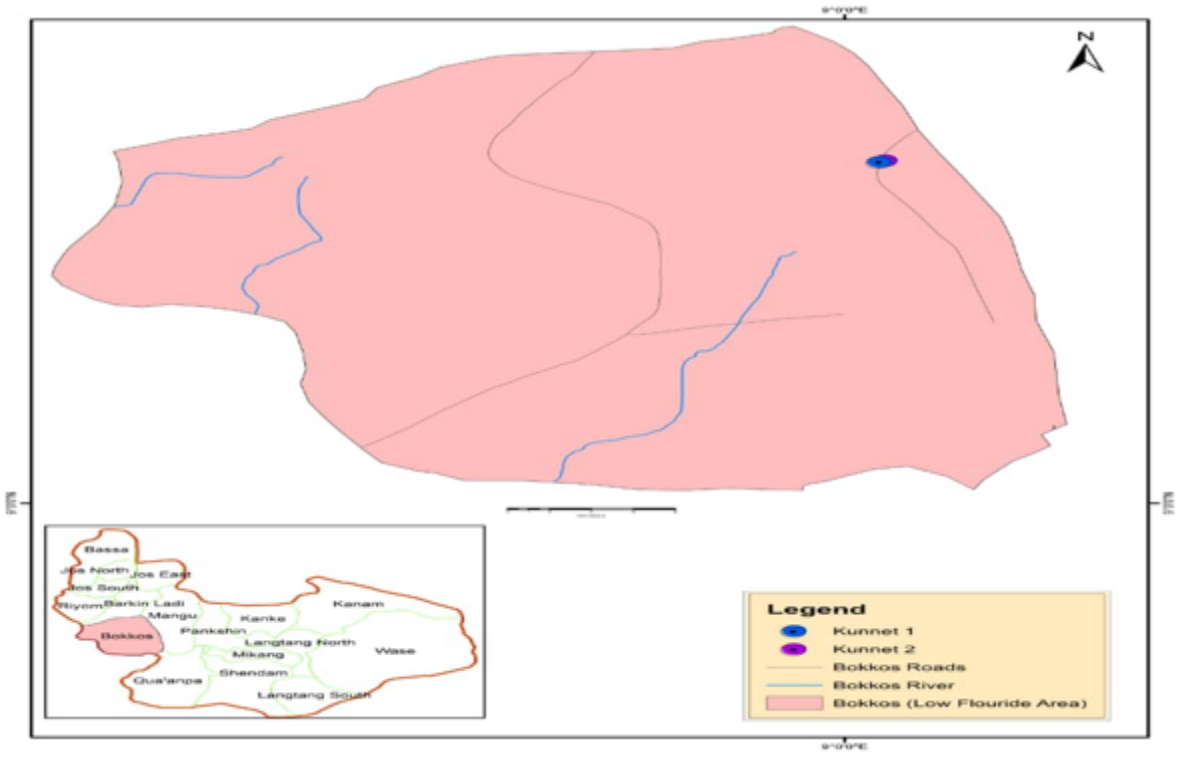

Figure 6.1A Map of Bokkos LGA (Low fluoride area)

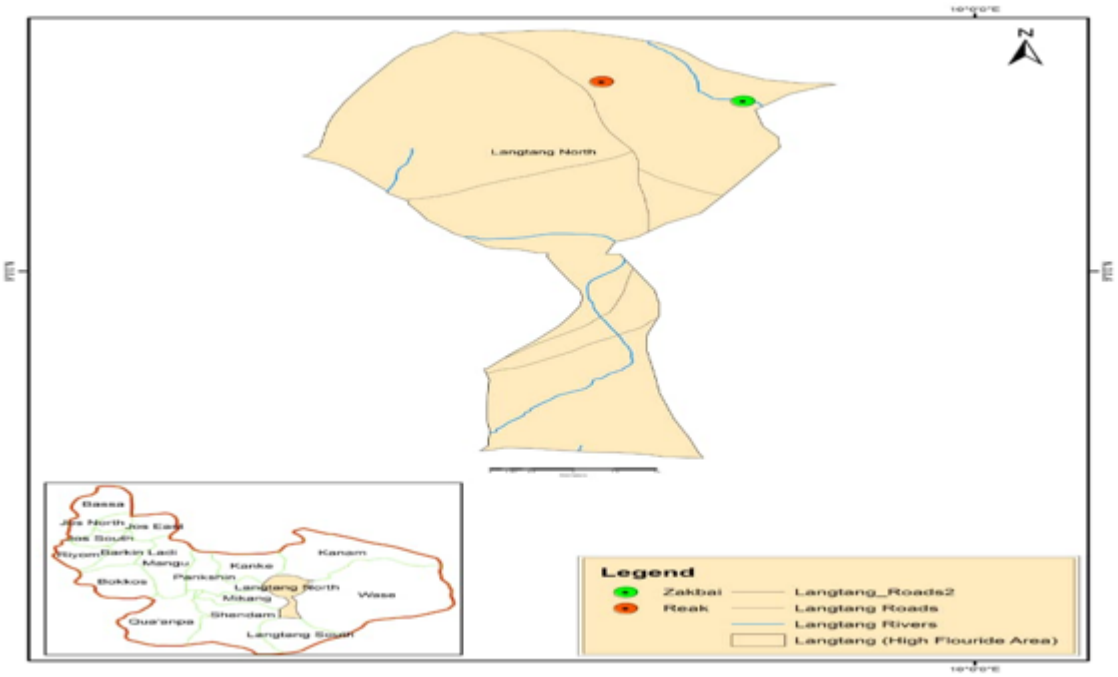

Figure 6.1B Map of Langtang LGA (High fluoride area)

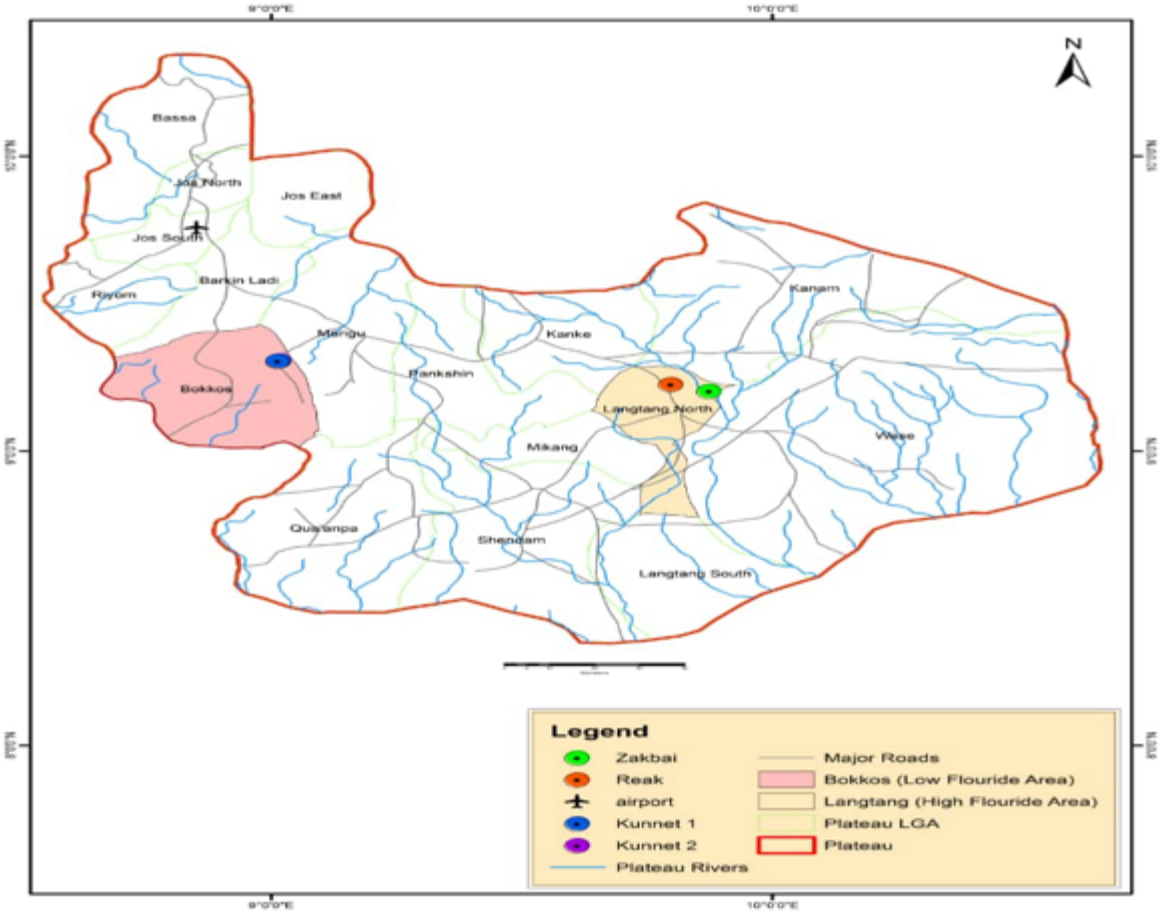

Figure 6.1C Map showing the study areas

6.4.1.1 Langtang North LGA

Langtang north LGA is located on the lowland of Plateau state between the 9°06' 16'' and 9° 10' 33''N latitudes and 9° 45' 18'' and 9° 49' 40''E longitudes and it spreads over an area of 2,476 km^2. It is bounded by Kanke, Mikang, Wase, and Langtang South LGAs in the north, west, east and south respectively. Langtang north LGA is situated close to the mountain range from the Jos Highland on the west with an altitude ranging from 500 to 1000 m above mean sea level. It has a semi-arid climate with maximum rainfall in August (296 mm) and driest between October and December. The hottest months are March and April with temperatures reaching 38°C and coolest by the end of December and early January with a fall in temperature to 18°C (Nanpon *et al*., 2014). According to the 2006 population census, Langtang north has a population of 111,148 people made up of indigenes known as Taroh.

6.4.1.2 Bokkos LGA

Bokkos is one of 17 LGAs in Plateau state with headquarters in the town of Bokkos at 9° 18' 00''N 9° 00' 00''E and spreads over an area of 1682 km^2. The total population of Bokkos LGA was 179,550 as recorded in the 2006 census. It lies in the region of Jos Plateau which has an average elevation of about 1250 m above sea level. Bokkos enjoys a tropical rainy climate with an average humidity of about 60% and heavy rains between June and August and mean annual rainfall figure is 146 cm and a mean annual temperature of 22°C with maximum temperature about 26°C and minimum 18°C. Generally, weather conditions are warmer during the rainy season (April - October) and much colder during the hamattan period (December - February) (Gontul, Oche, and Daloeng, 2007).

6.4.2 Ethical considerations

6.4.2.1 Ethics approval

Approval for the study was obtained from the School of Health and Social Care Ethics Committee, Teesside University (Study No 065/15) (see Appendix 2) followed by approval from Jos University Teaching Hospital Ethics Committee, JUTH, Plateau State, Nigeria (JUTH/DCS/ADM/127/XIX/6408) through submission of a detailed study protocol (see Appendix 4).

6.4.2.2 Approval from the Ministry of Health/Hospital Board Management

Upon positive approval from Teesside University and Jos University Teaching hospital, approval was also obtained from the Plateau State Ministry of Health and Hospital Board Management authority, Plateau State, Nigeria. Approval was given to collect human biological samples from volunteers including adults and children, to use the hospital facilities for processing and analysis of samples due to the sensitivity of the samples particularly blood, and to employ support staff including phlebotomist and laboratory assistant. A brief protocol of the study was given to the Ministry of Health as well as the Hospital Management Board, Plateau state to ensure that they understood the details of the study and to approve all aspects of the proposed work.

6.4.2.3 Approval from the Local Government Authority

Upon approval from the Ministry of Health and Hospital Board Management, the selected Local Government Authorities where the study was to be conducted were contacted and verbal approval was obtained prior to the commencement of the study to visit the selected villages and undertake the study.

6.4.2.4 Approval from District and Village heads

With the assistance of the LGAs, verbal consent was given by the District heads and heads of villages selected for the project. A meeting was held in the villages with the village head and chiefs representing the households in the communities and a representative from the LGA where the researcher ensured that all details about the study were provided and any question they had prior to the commencement of the study were answered. Verbal approval was given to visit selected villages and a representative from the council selected to monitor the whole process and organise a date when all households within the village could assemble for the study. The approval included the collection of the demographic information, tooth brushing habits, and collection of biological markers.

6.4.2.5 Permission from parents or legal guardians

With the assistance of the village head, parents or legal guardians of children aged 4-5 years were invited to a meeting at the community centre or village clinic after details of the research was communicated to them by the village head. The study pack including invitation letter for the parent, demographic data and tooth brushing habit form, medical history form,

participant information form, a separate information sheet for DNA collection, guideline for the collection of the samples was provided for each household. During the meeting, they were informed about the details of the study. The meeting was interactive and answers were provided by the researcher for all questions asked by the parents with the assistance of interpreters recruited for the study. Parents/legal guardians were given sufficient time (2 to 3 days according to the time to commencement) to think through prior to the acceptance of study and consenting to the participation of their child. Due to the level of education of parents, as most could not read and write, the interpreters were properly trained to communicate the research in local language and every detail of the consent was properly translated before parents assigned their signatures. Only the parents who consented to the study for themselves as well as for their child, participated in the study and participants were told they could withdraw from the study without any explanation or reasons.

6.4.3 Subjects and sample size

6.4.3.1 Subjects

The subjects were healthy children and their parents:

1. Age: Children aged 4-5 years and their parents aged ≥20 years.

2. Gender: Subjects from both genders.

3. Healthy volunteers who complied with the following inclusion and exclusion criteria:

Inclusion criteria: Volunteers aged 4-5 and ≥20 years old of either gender; with no dietary restriction; no chronic or metabolic disease and urinary infection; no oral disease (no tooth or gum pain) and no professional dental treatment such as the use of fluoride varnish for at least three months prior to the start of the study; parental consent for children as well as assent from the children, able to provide all the samples including blood, saliva, 24-hour urine, hair and nails.

Exclusion criteria: Volunteers who were using medication; had a restricted diet; had metabolic disease and/or urinary infection; had any oral disease (tooth or gum pain) and/or professional dental treatment such as the use of fluoride varnish for at least three months prior to the start of the study; had any nail disease; used hair dye; were unwilling to stop using nail varnish for the specified study period up to sample collection or were unwilling

to remove nail varnish for the specified study period up to sample collection; individuals with a history of a blood-borne pathogen (e.g. viral hepatitis, HIV/AIDs).

6.4.3.2 Sample size

A sample size of 120 was shared among the four groups (2 areas x 2 age groups) with each group having a sample size of 30. See Chapter 4, Section 4.4.4 for details.

6.4.4 Study plan

6.4.4.1 Preparation for data collection

Preparation in the United Kingdom: The study documentation, including questionnaires, urine data sheet, and biomarker collection sheet, was prepared in the UK and an electronic copy saved. The researcher received training on analysis of fluoride in food and drinks as well the biological markers including urine, saliva, plasma, hair and nails using a fluoride ion selective electrode. A detailed Gantt chart for the field work, containing the weekly as well as daily activities, was designed and approved by the supervisory team (Appendix 21). A list of equipment and chemicals required was put together and Teesside University procured those that could be taken along to Nigeria while others were purchased from commercial shops in Nigeria, particularly chemicals. Permission was also sought from the relevant authority to bring samples of hair, nail and DNA samples back to the UK for analysis. Research material was carefully packed and transported to Nigeria by the researcher.

Preparation in Nigeria: Consumables, including sample containers and chemicals, were procured from commercial shops in Nigeria. Hard copies of all supporting documents were printed and photocopies were made in preparation for field work. The visits were organised to the LGAs to be covered by the research and meetings organised with the authorities in the Local Government as well as with the Medical Director in the hospitals where some of the laboratory work will be conducted. An advertisement was placed in the hospital for the position of phlebotomist, whereas recruitment of interpreters was through the local government officials. One week training was organised for the recruited research staff where the details of the study were discussed, individual roles identified and questions were answered. Interpreters were made to interpret the supporting documents into the languages, Hausa and Tarok for low fluoride area and high fluoride area respectively, and back into English to ensure documents were properly translated. With the assistance of the

representative from the LGA, the district heads were visited and meetings were organised with the village chiefs, where the details of the research were discussed, questions were answered and a timeline was drawn up for the study in the villages. A laboratory for the analysis of the samples was secured, a storage facility was rented and a means of transportation was organised prior to the commencement of the study. The facility where DNA analysis was conducted (Plateau State Institute of Virology Research) was also secured.

6.4.4.2 Sampling method

This study was an analytical cross sectional study which measured total fluoride intake (diet and toothpaste), urinary fluoride excretion and fluoride retention in saliva, plasma, nails (fingernails and toenails) and hair of people living in high and low fluoride areas of Nigeria. The study was conducted between March 2016 and November 2016. Volunteers were children and their parents aged 4-5 and ≥20 years respectively. In view of the age ranges selected, targeting schools was considered the best approach for the recruitment of individuals. However, where there were no schools in the community for the age range of the children, participants were recruited among households within the selected villages in the Local Government Areas (LGAs). A LGA was randomly selected from Plateau central senatorial district for the low water fluoride area, as all the areas within this district had low water fluoride concentrations, while Langtang north LGA in Plateau south senatorial district was purposely selected for the high fluoride in drinking water. This was based on the results obtained from the preliminary study conducted in the region (Table 6.1). Information on the number of villages as well as their distribution within the randomly selected local government areas were obtained. The Plateau central senatorial district is made up of Bokkos, Kanan, Mangu, Kanke and Pakinshin LGAs; all with a low fluoride concentration in the water. Bokkos LGA was randomly selected and the sampling frame was all households within the LGA. Langtang South senatorial district is made up of Langtang north, Langtang south, Mikang, Quaán Pam, Shendan, and Wase. Langtang north was selected based on its high concentration of fluoride in the water. Two villages were purposefully selected and all the households were contacted to take part in the study.

6.4.4.3 Procedure for participants' recruitment

See Chapter 4 for details.

6.4.5 Data collection

6.4.5.1 Demographic data and anthropometric data

Demographic information including name, home address, and date of birth, gender, and the name of the village was collected. The height and weight of each volunteer (parent and child) were measured and recorded on the anthropometric data sheet during the introductory session after informed consent of the parents had been obtained. Height was measured using a portable stadiometer vertically without shoes to the nearest 0.5 cm and the weight was measured using a portable digital scale without shoes to the nearest 0.1 kg. The same weight and height scale was used throughout the study to avoid possible measurement error. The weight scale was calibrated using a standard 10 kg weight before use.

6.4.5.2 Food Frequency Questionnaire (FFQ)

Parents/guardians of children recruited were required to complete a Food frequency questionnaire designed for the study to explore the participant's dietary exposure (food and water). It contained questions relating to food consumption in addition to the type of water consumed. Dietary data were collected using the FFQ, which was completed by parent and child separately with the assistance of the researcher on the day of the visit to the participants' home. Parents were asked the food they consumed over the past few months and their frequency of consumption. They were also asked to use household measures such as spoons, cups and plates to estimate the amount of food and drinks consumed. Two samples of water (depending on the source) were collected from each family on the visit to the participant's home and kept in 7 ml bijou tubes. Participants were encouraged to recall all food as well as snacks consumed (type and amount recorded in the FFQ) by the child during the interview. The researcher ensured that all food and drinks were recorded in the FFQ and weight of food and drinks were properly estimated using household wares provided in the field shown in Figure 8.30/31. Five participants were selected randomly in each community representing the low and high fluoride areas to provide all samples of food and drinks consumed. They were given pre-labelled polystyrene containers and tubes and asked to provide a sample of approximately 5g of each homemade food and drinks consumed by themselves and their children, which were kept in a fridge until analysis. Purchased foods recorded in the FFQ were also obtained by the researcher for analysis.

Table 6.2: Summary of data collection procedure

Introductory session	Assessment of medical history
	Weight and height recorded
	Biomarker Acceptability Questionnaire (BAQ) completed
Home Visit	Home-made food and drinks consumed by parent and child recorded separately by the researcher
	Ready-made foods/drinks recorded in the FFQ obtained by the researcher
	Selected participants provided samples of homemade food
	Samples (food and drinks, 24-hr urine, saliva, plasma, fingernail, toenail, and hair) were picked up by the researcher
	Samples transported in cool box to the laboratory used for analysis (nail and hair samples transported at room temperature)
	Sample preparation and storage
	Sample analysis

6.4.5.3 Method of data collection – Oral hygiene Questionnaire

General information about the tooth brushing habits of both the parent and children was obtained during the introductory session. This included information on the frequency of brushing per day, the age of starting tooth brushing, type of regularly used toothpaste, the person (parent or child) who placed the toothpaste on the toothbrush. All information was recorded in the toothpaste data recording sheet to estimate fluoride ingestion from toothpaste. Specific information about the toothpaste, including: brand name, flavour, type of toothpaste (parent and child), the form of fluoride in the toothpaste (sodium fluoride, sodium monofluorophosphate or a mixture of both), and fluoride concentration of toothpaste (ppm) from the manufacturers' labelling, were recorded.

6.4.5.4 Data on the acceptability of biomarker

An initial Biomarker Acceptability Questionnaire was completed during the introductory session. On the day of visit, after the collection of blood had been completed by the qualified nurse, participants were given a final fluoride biomarker acceptability questionnaire which was completed by the participant with the assistance of the researcher and collected by the researcher on the same day.

6.4.6 Sample collection, preparation and analysis

6.4.6.1 Sample collection

- **Drink and food samples**

Home-made drinks and foods

After parents/guardians were interviewed on their food and drink consumption as well as their child's, the researcher identifies the foods and drinks that were frequently consumed from the FFQ and made a list which was passed to a cross-section of parents (5). The researcher gave them universal tubes and polystyrene bowls labelled with food/drink and date of preparation. The selected parents prepared the foods and drink in their usual way and provided a serving spoon of home-made food and a half tea cup of any home-made drink consumed in polystyrene bowls and universal tubes respectively. Samples were picked up by the researcher on an arranged date and transferred to the Federal College of Forestry, Jos Plateau state Nigeria for immediate analysis. Each home-made food and drink sample provided by the parents were collected together based on the type of food and drinks. Each sample was then homogenised using a blender and divided into 2 aliquots. One aliquot of food and drink was analysed for fluoride and the other aliquot was stored by the researcher at -20^{o}C in the Federal College of Forestry, Jos Plateau State, Nigeria.

Ready-to-drink and ready-to-eat samples

After the questionnaire had been completed by the parents, a list of the frequently consumed ready-to-drink and ready-to-eat samples was extracted from the questionnaire by the researcher, who then purchased the items from commercial stores in Jos municipality of Plateau state. Each of the purchased food and drinks was divided into two (a portion for analysis of fluoride and the other stored at -20^{o}C) and placed in polystyrene bowl and universal tubes respectively.

- **Toothpaste samples**

Toothpaste that was used by the participants who brushed their teeth were identified from the "Fluoride Exposure Questionnaire" completed by parents/legal guardian and a list was derived consisting of five kinds of toothpaste: Close-up (deep action), Close-up (herbal), Oral B, Colgate, and Macleans. These brands of toothpaste were purchased in the

commercial stores in the study area and stored at room temperature in the Federal College of Forestry, Jos prior to transport to the UK for fluoride analysis.

- **Biomarker sample collection**

All biomarker samples were provided by the parents as well as their children (See methods of collection of each biomarker in Chapter 4).

6.4.6.2 Sample preparation

For details see sample preparation for biomarkers as well as food and drink samples in Chapter 4.

6.4.6.3 Transportation of samples

Collected blood and tissue samples were sealed inside a waterproof box and labelled properly (name of the sample, date of collection and identity code). The researcher ensured appropriate packing of the blood samples to absorb spillage if the vials leaked. Samples were then transported in the researcher's private car from the participant's home to the chemistry laboratory of the Federal College of Forestry, Jos, Nigeria where some of each sample was stored and later analysed. The waterproof box was kept in the boot of the car during transportation to the laboratory.

6.4.6.4 Fluoride analysis

Water, non-milk-based drinks and urine samples were analysed by a direct method using a fluoride ion specific electrode (Taves, 1968, Martinez-Mier *et al*., 2011). Food samples, milk-based drinks, blood, saliva, hair and nail samples were analysed by an indirect method using a modification of the hexamethyldisiloxane (HDMS) method (Whitford 1996). Urine, plasma, saliva, water, expectorated saliva/rinses samples and food samples were analysed in triplicate by the researcher in the Chemistry laboratory of the Federal College of Forestry (FCF), Jos, Plateau State, Nigeria and leftovers were discarded appropriately. Hair and nail (fingernail and toenail) samples were brought to the UK and analysed for fluoride in the fluoride laboratory, School of Health and Social Care, Constantine building laboratory (C1.11), Teesside University. DNA was extracted from the blood samples in the DNA laboratory of Plateau State Institute of Virology, Jos, Plateau State, Nigeria and extracts were transported to the UK, for investigation of genes regulated by fluoride in the body at Newcastle University cell biology laboratory. Permission was obtained from the relevant

regulatory agency and transport company and then samples of hair, nails and DNA extract were sealed in a waterproof box and labelled appropriately (name of sample, date of collection and identity code) according to the agency's guidelines before transporting to the UK. All food samples were also analysed for fluoride in Nigeria.

6.4.6.5 Disposal of samples

Urine samples that were left over were disposed of in an allocated toilet. All saliva, blood, nail and hair samples collected were used for the analysis. Bijous, containers and bottles containing urine were placed in Virkon solution (1%) for two hours prior to disposal. The containers were rinsed and placed in yellow bags, labelled as clinical waste and disposed of according to Federal College of Forestry, Jos disposal systems. Disposable gloves and plastic aprons were put in clinical waste bins (yellow, labelled bin liners) for incineration. Disposable plastics including tubes, pipette tips, culture flasks, etc. was decontaminated and then put in clinical waste bins (yellow, labelled bin liners) for incineration. Disposable glassware as well as blood sample bottles were also decontaminated before being put in clinical waste bins (yellow, labelled bin liners) for incineration. In case of excess human material (tissue (hair and nail), blood plasma), they were decontaminated and then put into clinical waste bins (yellow, labelled bin liners) for incineration. All the above were finally disposed of according to the Federal College of Forestry, Jos disposal system.

6.4.7 Data preparation and analysis

Raw data were recorded in an Excel spreadsheet upon completion and a summary file containing the main variables was generated. Descriptive statistics were generated using the Statistical Package for the Social Sciences (SPSS) version 22. Descriptive statistics were used to summarise the data sets. Correlation analyses were used to find the relationships between fluoride intake and Daily urinary fluoride excretion, fluoride concentration in plasma, fluoride concentration in saliva, fluoride concentration in fingernail/toenails, and fluoride concentration in hair. Similarly, a relationship between fluoride concentration of drinking water and concentration of fluoride in the biomarkers was in investigated. Regression analysis were used to find the relationship between fluoride concentration of the biomarkers and independent variables including age and fluoride area.

CHAPTER 7: NIGERIA QUALITATIVE STUDY

7.1 INTRODUCTION

This Chapter is the second part of the study conducted in Nigeria and comprises the qualitative aspect. The chapter begins with the aim and objectives of the qualitative study, followed by the methodology used, then the results (generated from the answers provided on the questionnaires) are presented. Finally, a discussion of the results is given, followed by the conclusions.

7.2 AIM AND OBJECTIVES

7.2.1 Aim

The main aim of this part of the work was to investigate the preferences for biological markers of fluoride exposure in children (4-5 years) and adults (≥20 years) in Nigeria using questionnaires.

7.2.2 Objectives

- Investigate the most acceptable biomarker of fluoride exposure by age group (4-5 and ≥20 years) using questionnaires;
- Evaluate the feasibility of collecting biological markers of fluoride exposure by age group (4-5 and ≥20 years) in Nigeria;
- Evaluate the willingness of participants to provide any of the biological markers among the age groups (4-5 and ≥20 years).

7.3 METHODS

This study utilised a qualitative approach, similar to the UK study, as described in Chapter 4 (4.3.5) and in more detail in Chapter 5 (Section 5.3.4).

7.4 RESULTS

7.4.1 Socio-demographic characteristics of participants

In total, 120 participants took part in the study: 61 parents and 59 children. Parents of 2 children did not complete the questionnaire on behalf of their child.

Table 7.1 illustrates the socio-demographic data of the parents who completed the questionnaires in the low- and high-fluoride water areas. The results show that more females (60.5%) participated than males. The majority of the participants were in the age groups of 25-34 years (53.2%) and 35-44 years (29.1%). In total, 80.3% (n=45) of the participants had a job, which was predominantly farming. A total of 56.4% were educated to GCSE/O-level, whereas 22.6% had no qualification.

Among the children, the percentages of males and females who participated in the study were similar and their mean (SD) age was 4.43 (0.72).

Table 7.1 Socio-demographic characteristics of parents

		Number	Percentage (%)
Gender	Male	24	39.5
	Female	37	60.5
Age range	18-24	5	8.1
	25-34	33	53.2
	35-44	18	29.1
	45-54	3	4.8
	More than 55	2	4.8
Do you have a job?	Yes	49	80.3
	No	12	19.7
Highest level of education	GCSE/O-level	35	56.4
	A-level/Diploma	3	4.9
	University Degree	1	1.6
	Vocational qualification	9	14.5
	No qualification	13	22.6

7.4.2 Adults

7.4.2.1 Attitude towards using the biological markers

Considering the attitude of the participants to how they felt about 24-h urine, which is currently the most frequently used biomarker, most of the respondents (89%) found collection to be either perfectly acceptable or slightly acceptable and a combined 15% of those surveyed said they found the collection totally unacceptable, unacceptable or slightly unacceptable (Figure 7.1). Similarly, when the same participants were asked how they felt about the way two-day spot urine samples were collected, 95% found it perfectly or slightly acceptable. The participants felt indifferent when they were asked if the spot urine was collected in a day, as 92% found it perfectly or slightly acceptable. Only 2% of the participants found both one and two-day spot urine slightly unacceptable (Figure 7.2).

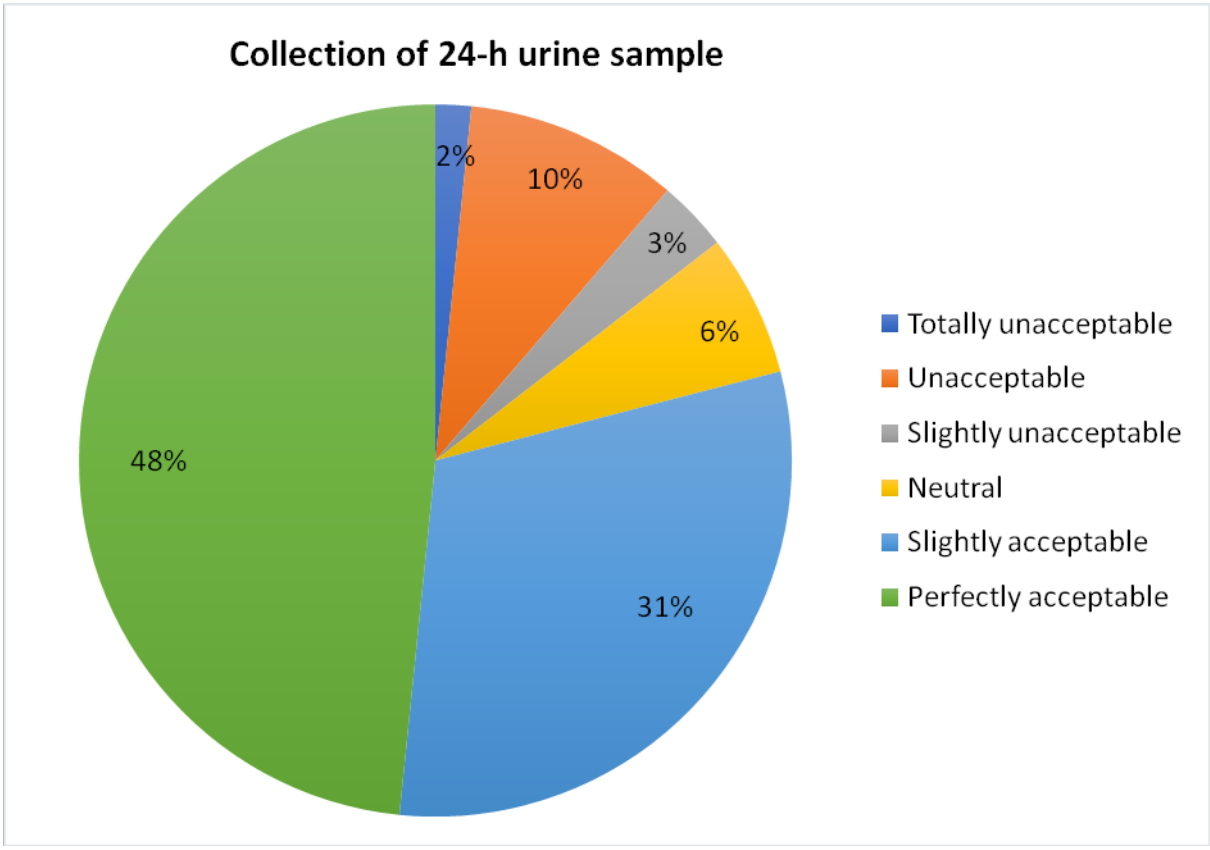

Figure 7.1 What do you feel about having to collect your urine for 24-h?

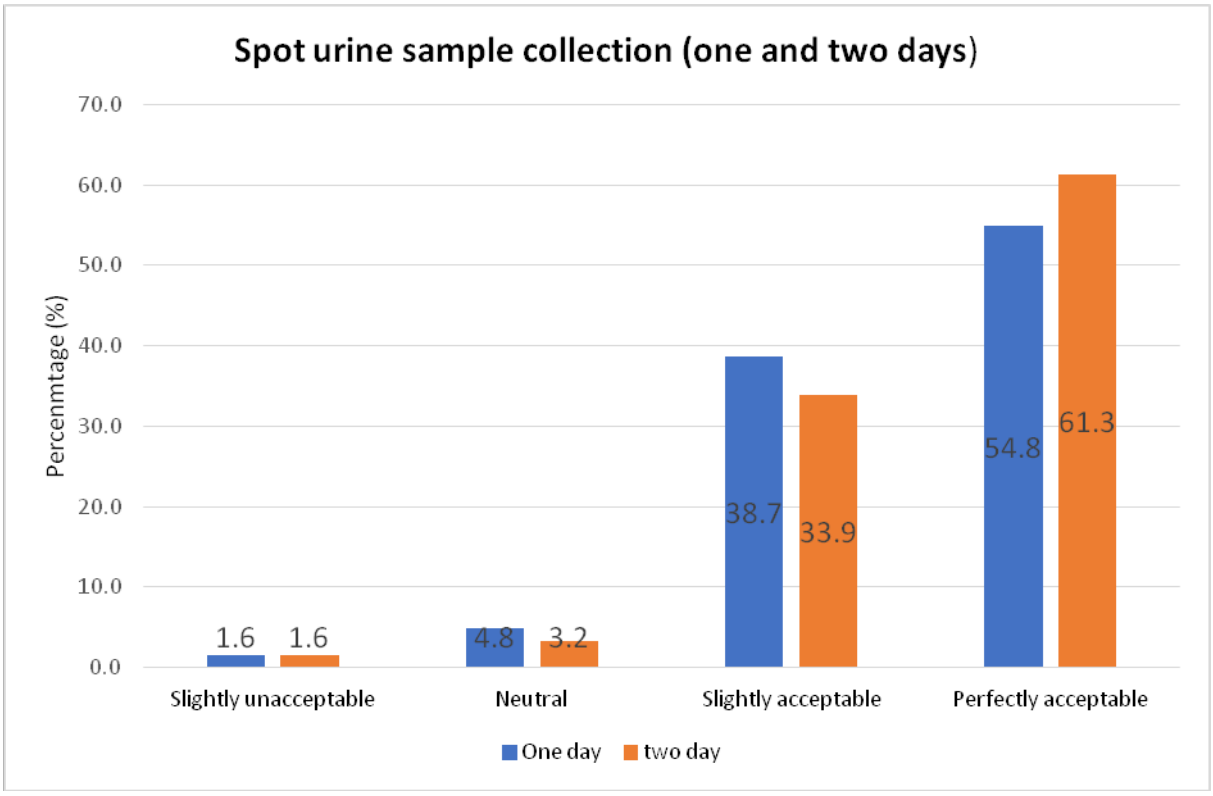

Figure 7.2 How do you feel about having to collect spot urine samples?

Considering the attitude of participants towards all the other biomarkers, including saliva, blood, nail and hair samples, the results show that there was no difference in their choice when asked about collection of either 24-h urine or spot urine samples. Overall, 61%, 60%, 60% and 64% said they found the collection of saliva, blood, nails and hair, respectively, perfectly acceptable. Likewise, a combined 95%, 92%, 87%, 87% found the collection of the samples of saliva, blood, nails and hair, respectively, either perfectly acceptable or slightly acceptable (Figures 7.3, 7.4, 7.5 and 7.6, respectively). These close percentages show that participants did not show any preference for a sample but a slight drop in the percentages to 87% when they were asked about how they felt about the collection of nails and hair samples showed that they would prefer the plasma and saliva compared to provision of nail and hair samples.

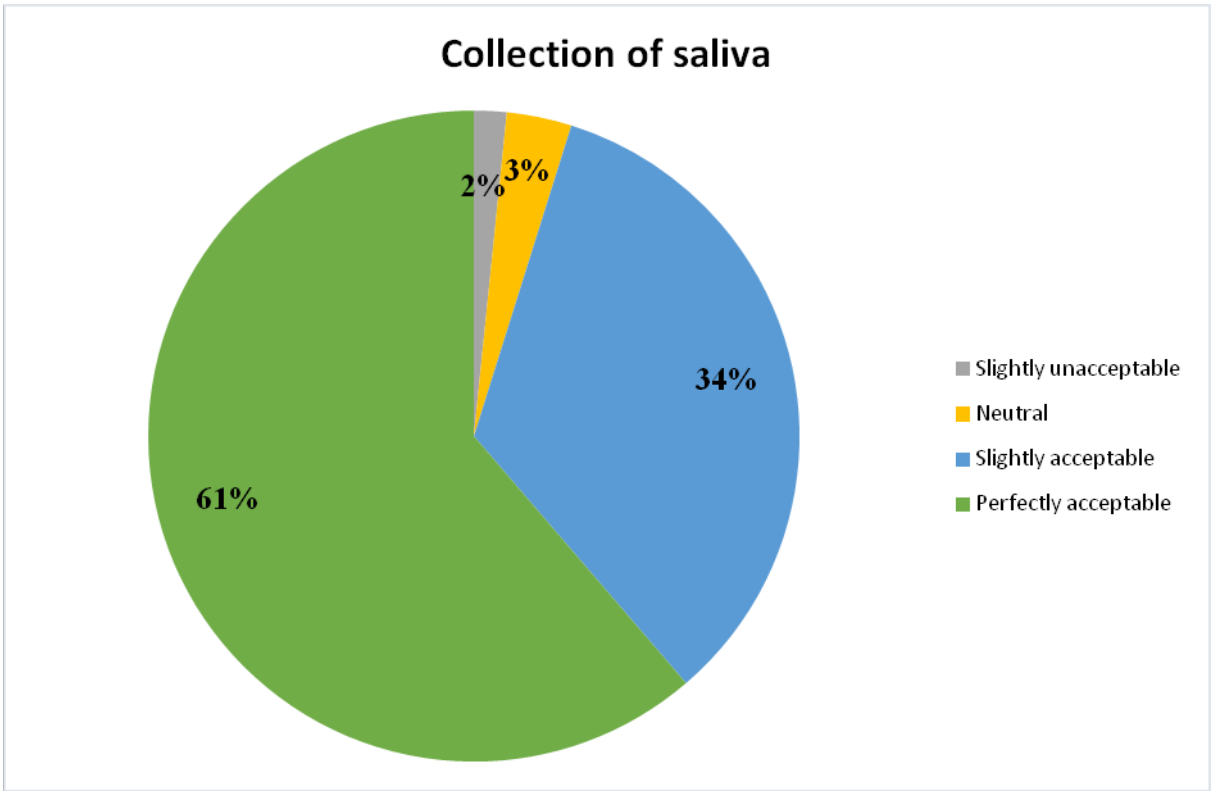

Figure 7.3 How do you feel about the saliva collection?

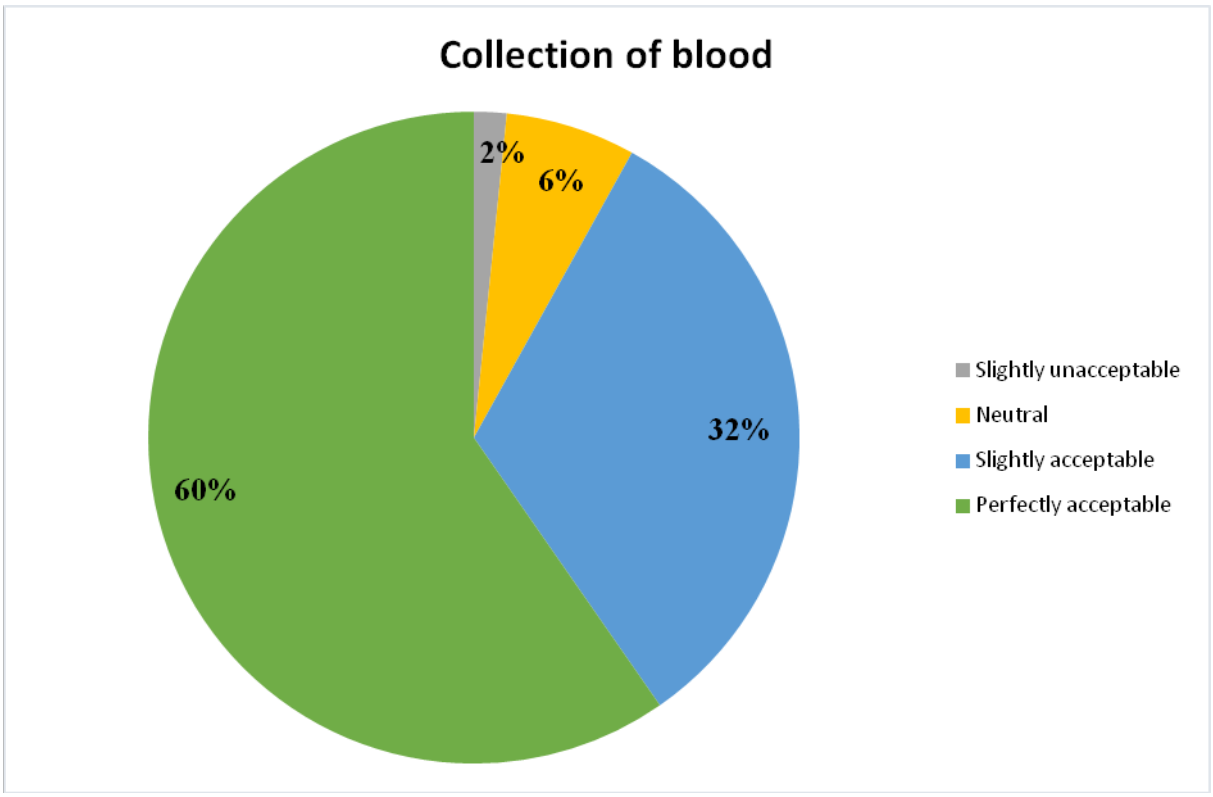

Figure 7.4 How do you feel about the way blood is taken?

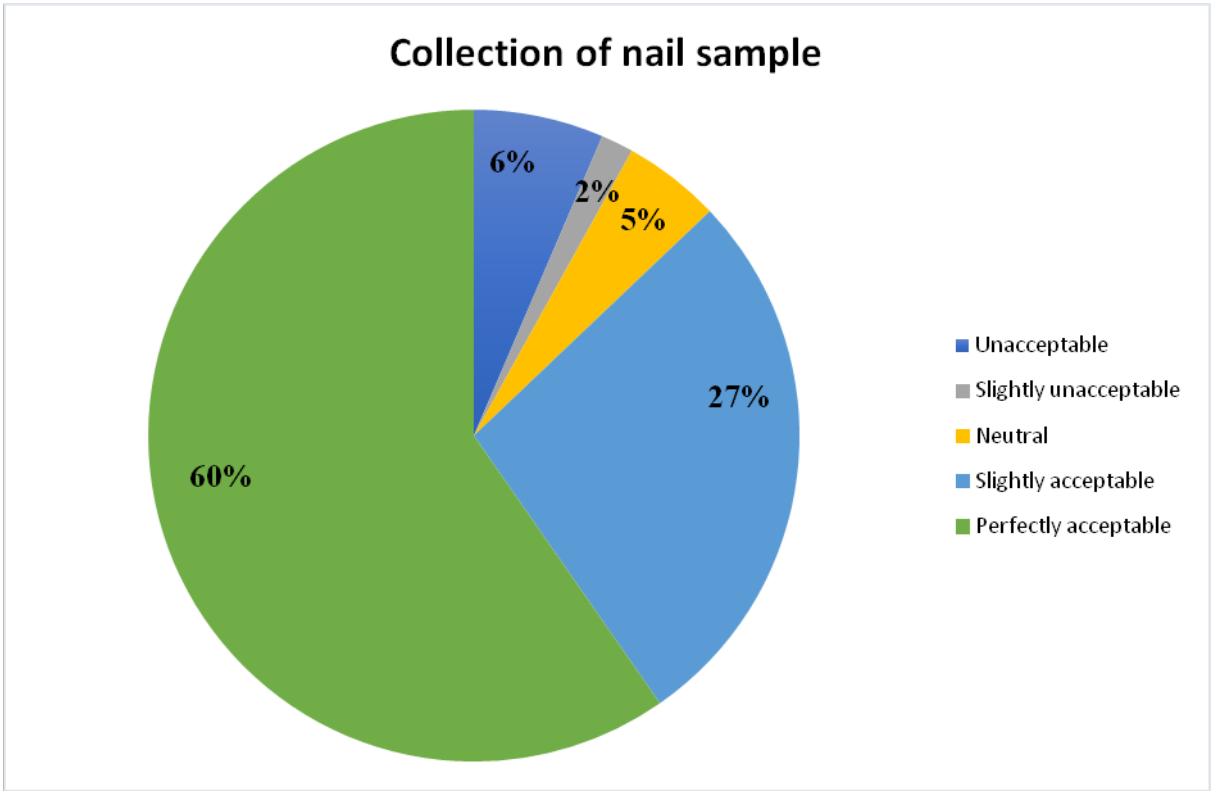

Figure 7.5 What do you think about being asked to collect your nail samples?

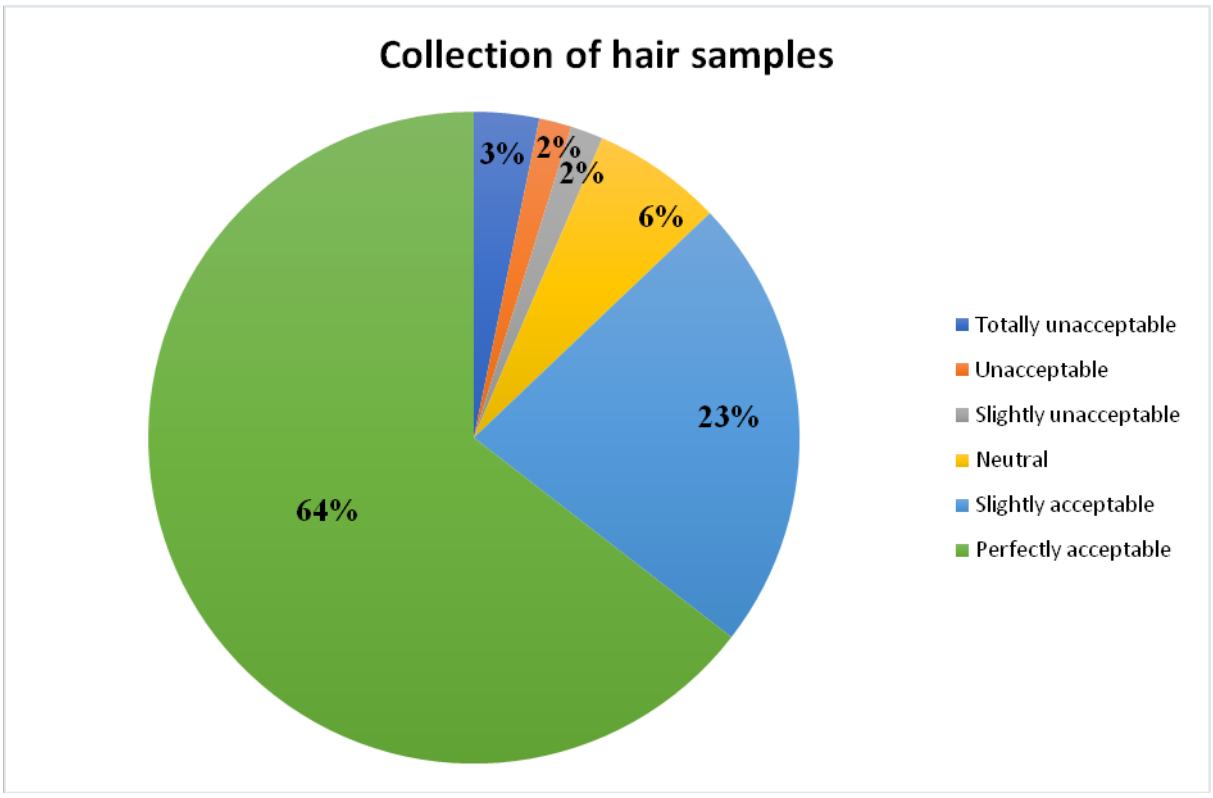

Figure 7.6 What do you think about being asked to collect your hair?

7.4.2.2 Perceived ease of collection of the biological markers

The results in Table 7.2 show the means for how participants would find collecting the biomarkers when asked to provide them. The range of the means is 8-9, showing that the participants would find it easy providing all the biomarkers. This was also confirmed by the value of the mode, as 10 (very easy) was the most selected value for all the biomarkers. However, there were slight differences in how easy they would find the collections. Most of the participants found saliva very easy (9.10), compared to blood (8.81), nails (8.69), hair (8.55), spot urine (8.28) and 24 h urine (7.98) which was not as easy.

Table 7.2 Ease of biomarker collection by adults (n = 62) in answer to the question: *Now that you know what you would have to do to collect……. Can you answer on a scale of 1-10 by selecting a number how you would find the collection of …… 1 means very hard and 10 means very easy?*

Biomarker	Mean	Median
24-hour urine	7.98	9.00
Spot urine	8.28	8.00
Saliva	9.10	10
Blood	8.81	9
Nails	8.69	9
Hair	8.55	9

7.4.2.3 Behavioural interest in the use of the biological markers

According to Figure 7.7, participants were more likely to provide either saliva or spot urine samples (98.4%) in the future, as they show the highest percentage acceptances, followed by either nails or blood samples, also with the same percentage (96.8%), 24 h urine (91.9%) and hair samples (90.3%). Overall, the results from Figure 7.7 show that most participants were likely to provide all the biomarkers considering that more than 90% chose “yes”. However, they were more likely to provide some than others. Contrary to their earlier choice, Table 7.3 indicates that blood is the most preferred (mode = 6) and it has the highest mean value (4.37), and the least preferred could be either 24 h urine or spot urine (mode = 1).

However, if we rank the biomarkers based on the means, spot urine would be the least preferred (2.73) by the adult group.

Table 7.3 Behavioural interest to the use of biomarkers: *Please rank in order of 1-6 by selecting a number how you prefer it. 1 means least preferred and 6 means most preferred.*

Biomarker	N	Mean	Median	Mode
24-hour urine	61	3.23	3.00	1
Spot urine	61	2.73	2.00	1
Saliva	61	3.97	4.00	4
Blood	61	4.37	5.00	6
Nails	61	3.15	2.00	2
Hair	61	3.52	3.00	3

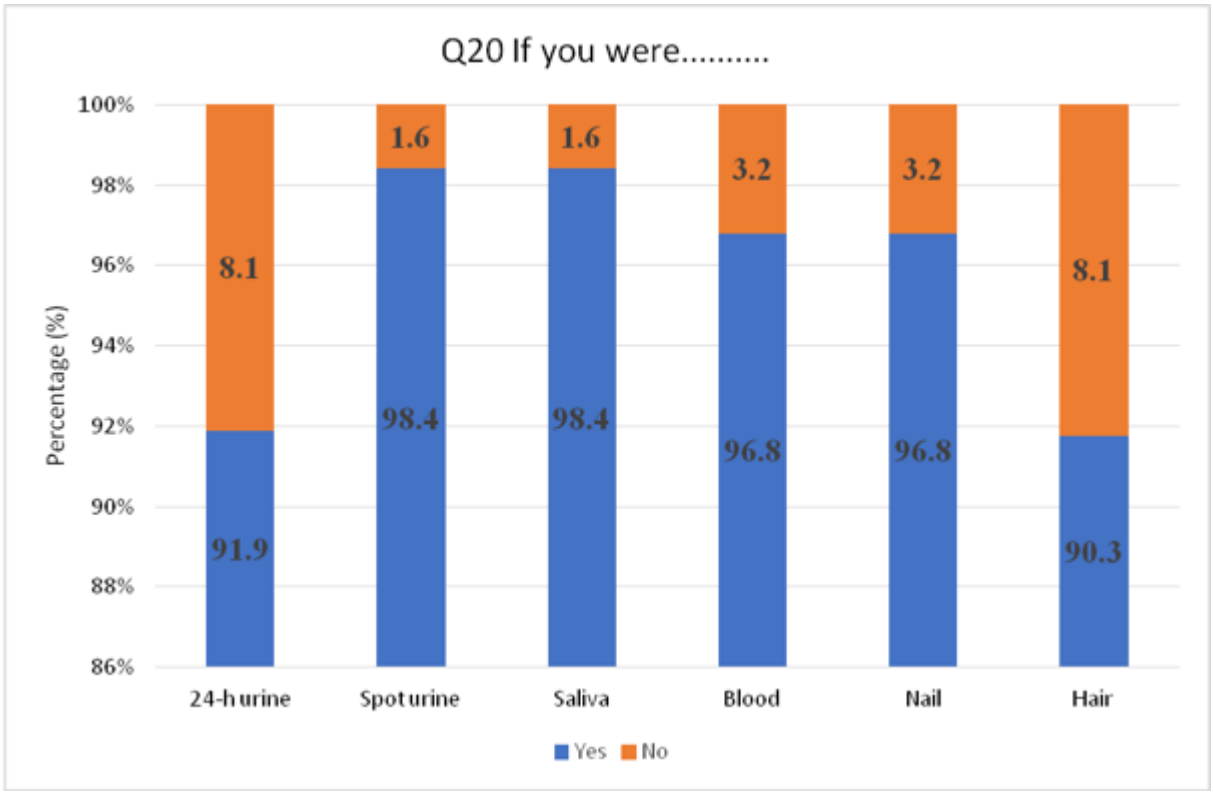

Figure 7.7 Willingness to provide biomarkers in future: *If you were asked to provide any of the above types of sample, which would you be willing to provide?*

7.4.3 Children

7.4.3.1 Attitude towards using the biological markers

The parents seemed indifferent about the collection of the biomarkers from their children as shown in Figures 7.8-7.13. In total, 66% of the participants found the collection of urine from their children to be perfectly acceptable, with a sharp increase in the percentage to 82% when asked how they felt about the collection of a spot urine sample for a two-day period. Surprisingly, there was a slight drop in the percentages to 68% when they were asked about a one-day collection of a spot urine sample from their children. Similarly, 81% of the participants found the collection of saliva samples from their child to be perfectly acceptable. A total of 93% of the participants felt the collection of blood from their child either perfectly acceptable or slightly acceptable, whereas only 2% felt the collection would be slightly unacceptable.

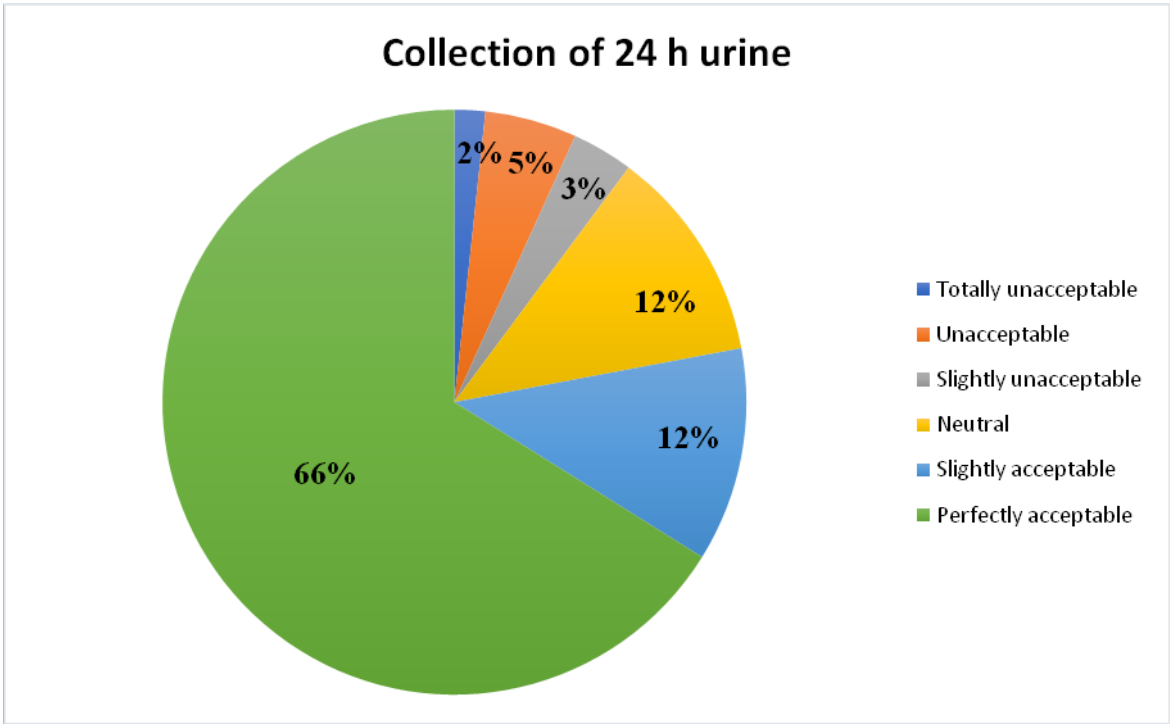

Figure 7.8 What do you feel about having to collect your child's urine for 24-h?

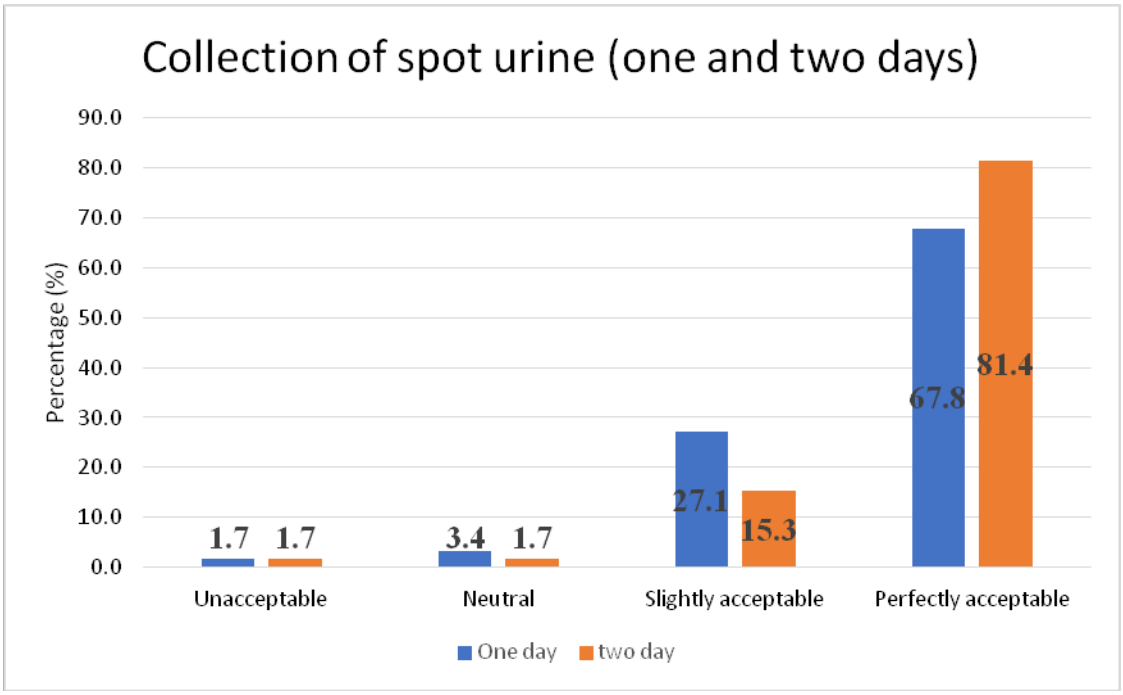

Figure 7.9 What do you feel about having to collect your child's spot urine samples?

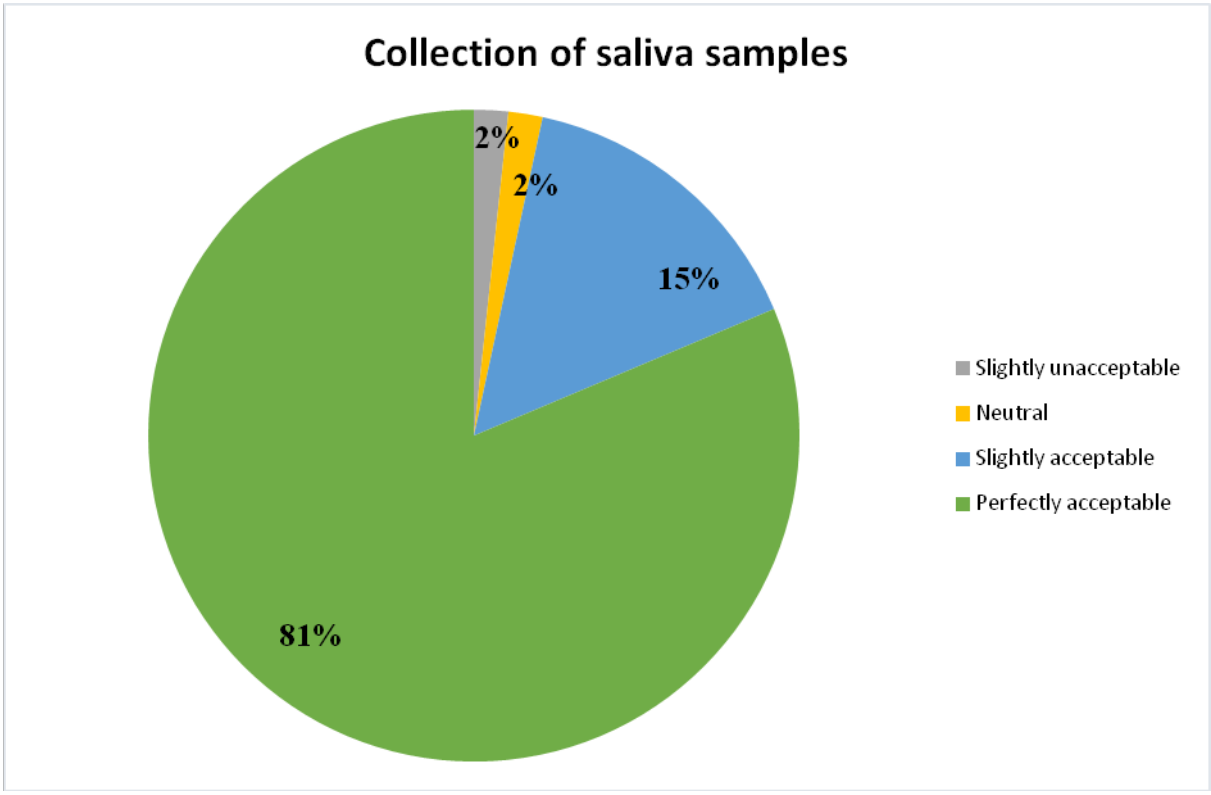

Figure 7.10 What do you feel about the saliva collection from your child?

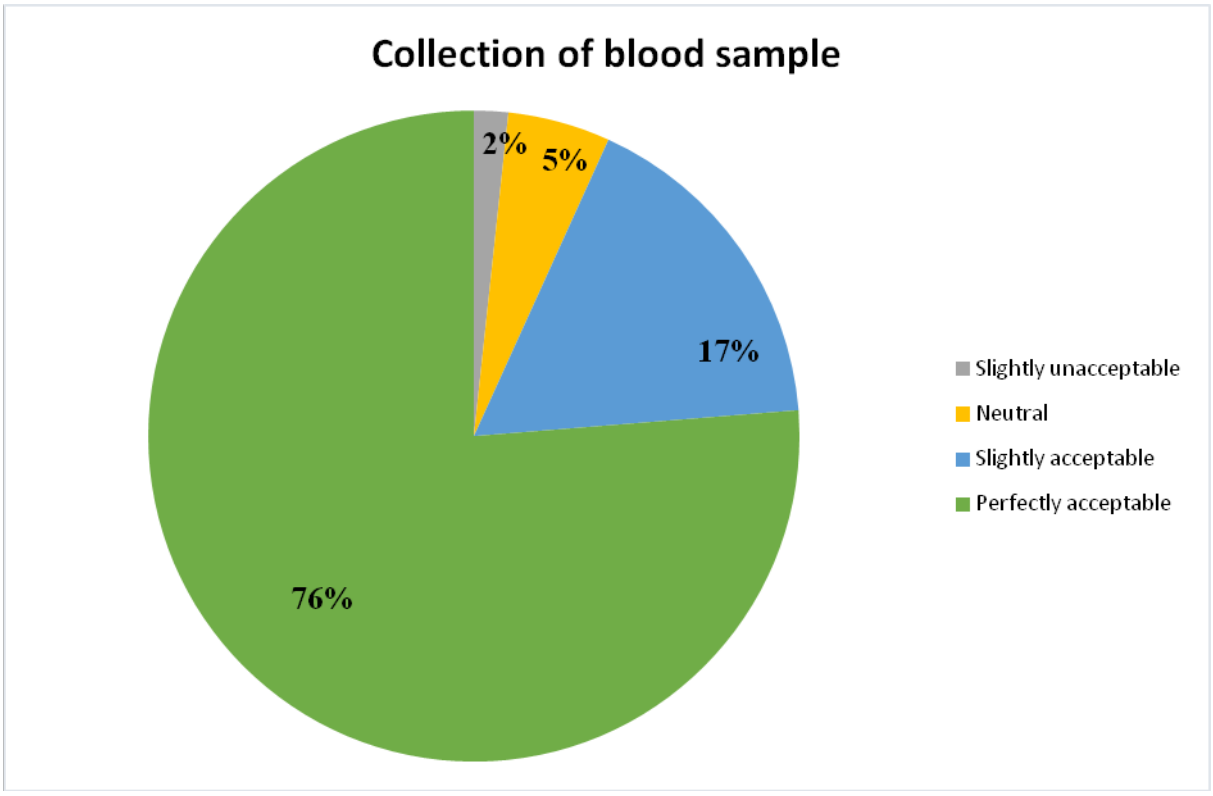

Figure 7.11 How do you feel about the way your child's blood would be taken?

Considering the participants' level of acceptance of the contemporary biomarkers urine, saliva and blood, they seem not to have had a different opinion about the recent markers, nails and hair, when asked about what they felt about the way the samples were collected. A combined 88% and 81% found the way samples of nails and hair, respectively, would be collected either perfectly acceptable or slightly acceptable. Meanwhile, 3% found the nail collection from their children to be unacceptable.

In summary, a combined 88%, 97%, 96%, 96%, 93%, 88%, and 81% found the way samples of 24 h urine, spot urine (one day), spot urine (two days), saliva, blood, nails and hair, respectively, would be collected from their children either perfectly acceptable or slightly acceptable.

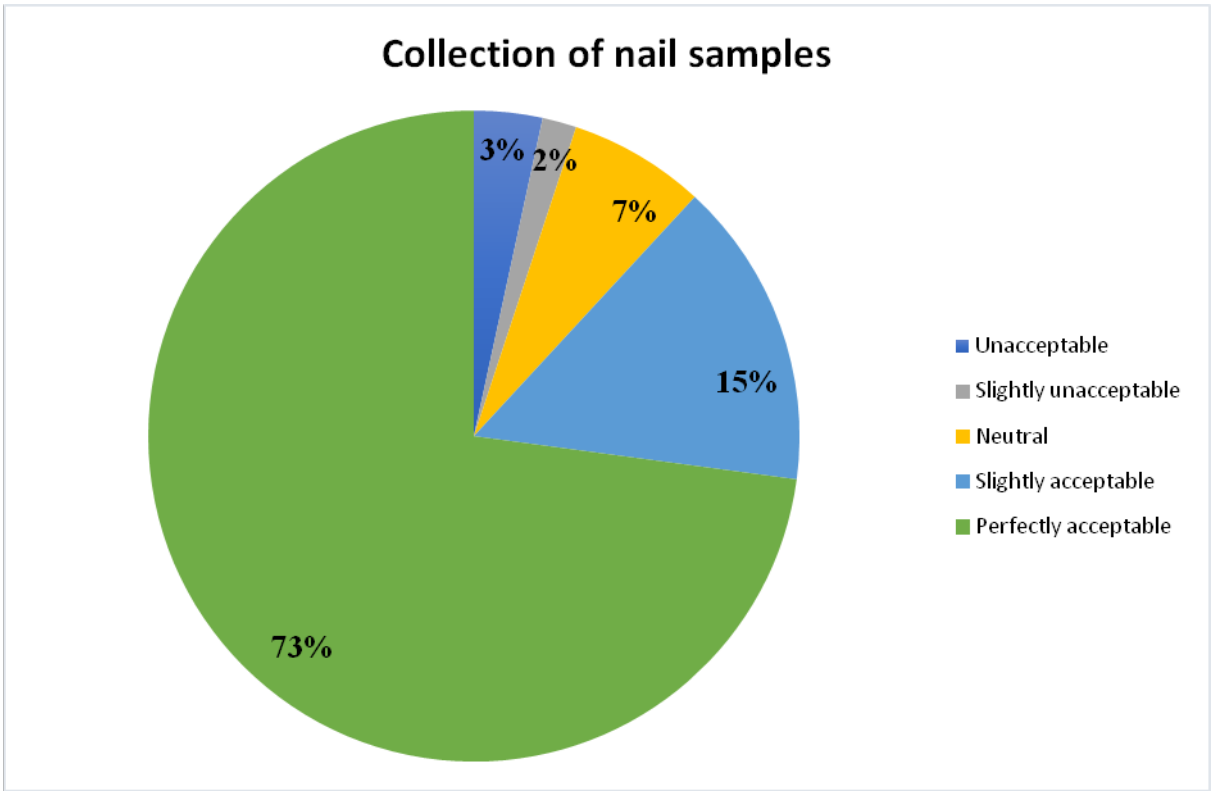

Figure 7.12 What do you think about being asked to collect your child's nails?

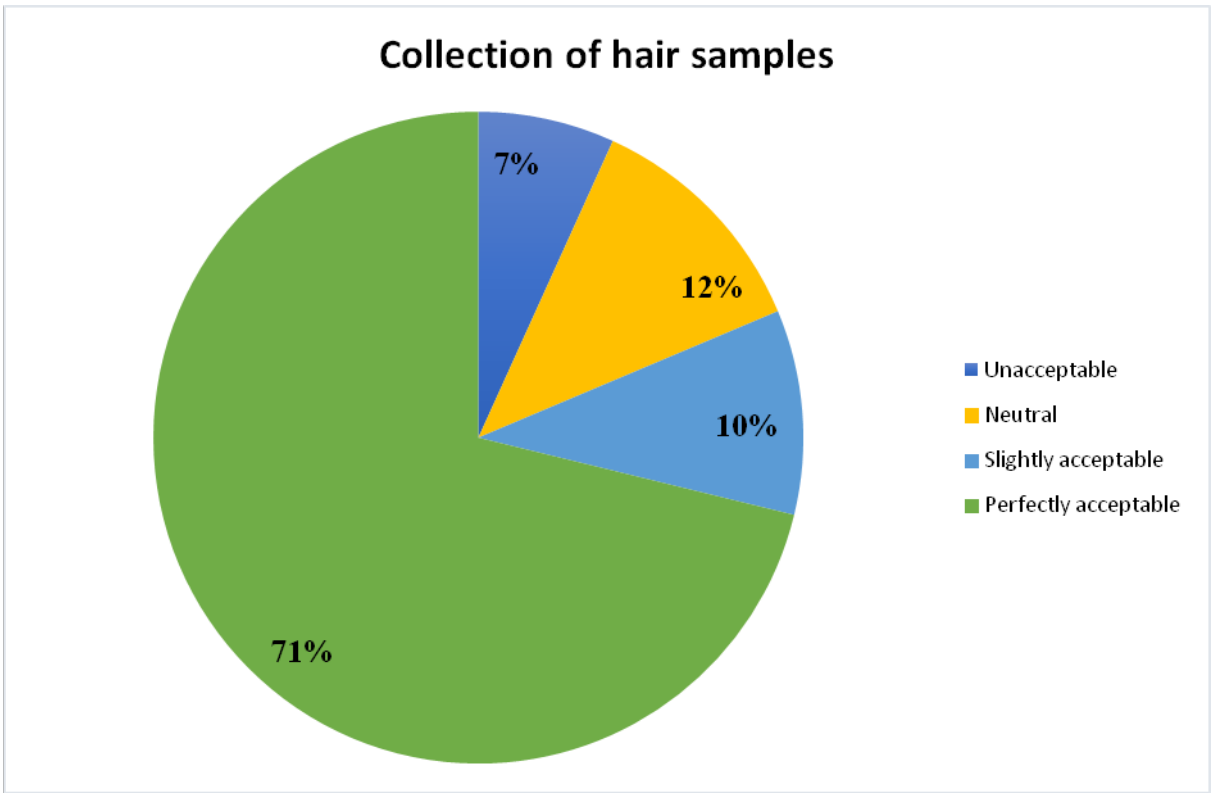

Figure 7.13 What do you think about being asked to collect your child's hair?

7.4.3.2 Perceived ease of collection of the biological markers

The results in Table 7.4 show the means for how participants would find collecting the biomarkers when their child was asked to provide them. The range of the means is 8-9, showing that the participants would find it easy providing all the biomarkers. This was also confirmed by the value of the mode, as 10 (very easy) was the most selected value for all the biomarkers. However, there were slight differences in how easy they would find the collection. Most of the participants found saliva very easy (9.42), compared to nails (9.22), blood (9.12), hair (8.88) and spot urine (8.28). However, they were indifferent between 24 h urine and spot urine samples.

Table 7.4 Ease of biomarker collection from children (n = 59) by their parents in answer to the question: *Now that you know what you would have to do to collect……. Can you answer on a scale of 1-10 by selecting a number how you would find your child collection of …… 1 means very hard and 10 means very easy?*

Biomarker	Mean	Median
24-hour urine	8.47	10
Spot urine	8.47	9
Saliva	9.42	10
Blood	9.12	10
Nails	9.22	10
Hair	8.88	9

7.4.3.3 Behavioural interest in the use of the biological markers

According to Figure 7.14, participants were more likely to collect either saliva or spot urine samples (98.3%) from their child in the future, as they show the highest percentage acceptances, followed by either nails or blood samples, also with the same percentage (94.9%), 24 h urine (91.5%) and hair samples (89.7%). Overall, the results from Figure 7.14 show that most participants were likely to provide all the biomarkers considering that more than 85% chose “yes”. However, they were more likely to provide some than others. Contrary to their earlier choice, Table 8.5 indicates that blood is the most preferred (mode

= 6), and it has the highest mean value (4.85), and the least preferred (mode = 1) is the spot urine sample with the lowest mean value (1.97).

Table 7.5 Behavioural interest in the use of biomarkers: *Please rank in order of 1-6 by selecting a number how you prefer it. 1 means least preferred and 6 means most preferred.*

Biomarker	N	Mean	Median	Mode
24-hour urine	59	3.36	3.00	2
Spot urine	59	1.97	1.00	1
Saliva	59	3.90	4.00	3
Blood	59	4.85	6.00	6
Nails	59	3.25	3.00	2
Hair	59	3.69	4.00	3

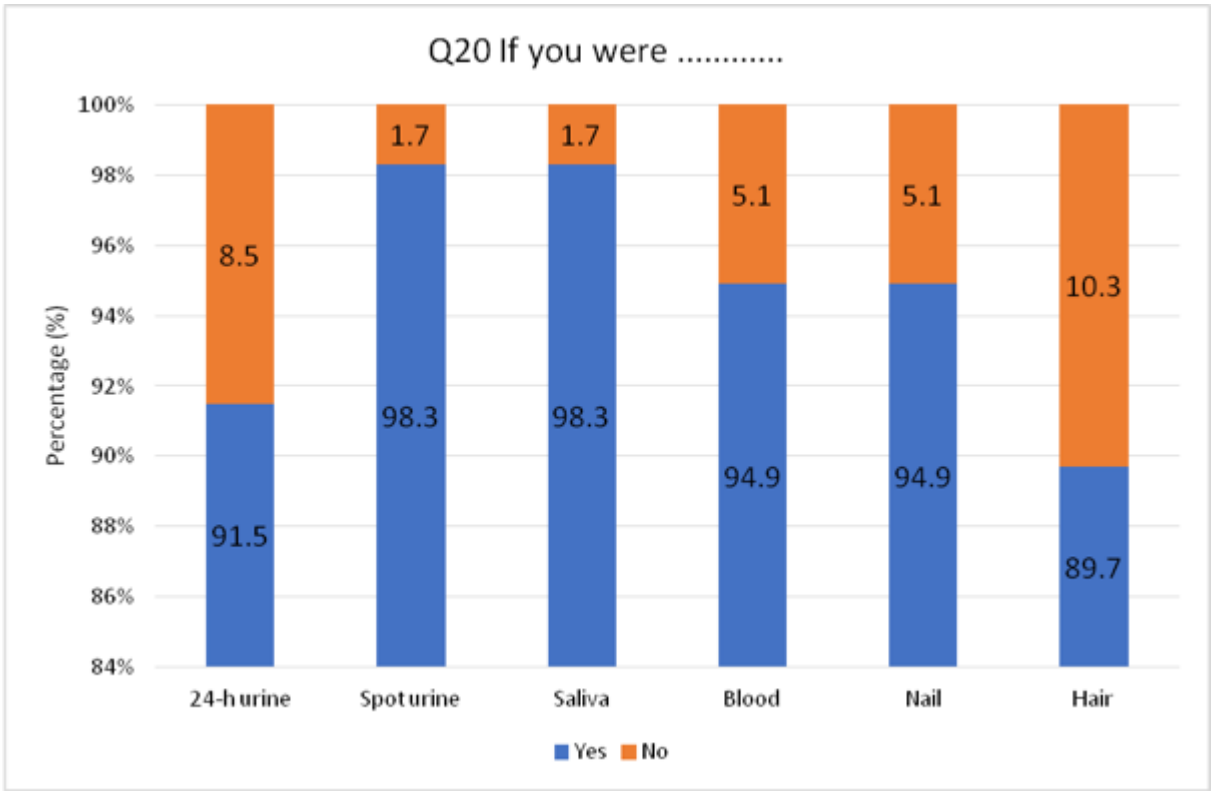

Figure 7.14 Willingness to provide biomarkers in future: *If your child was asked to provide any of the above types of sample, which would you be willing to provide*

7.4.3.4 Comparing acceptability of biomarkers between children and adults

Figure 7.15 shows that there was no notable difference in perception about the collection of biomarkers of exposure from the adults and their children.

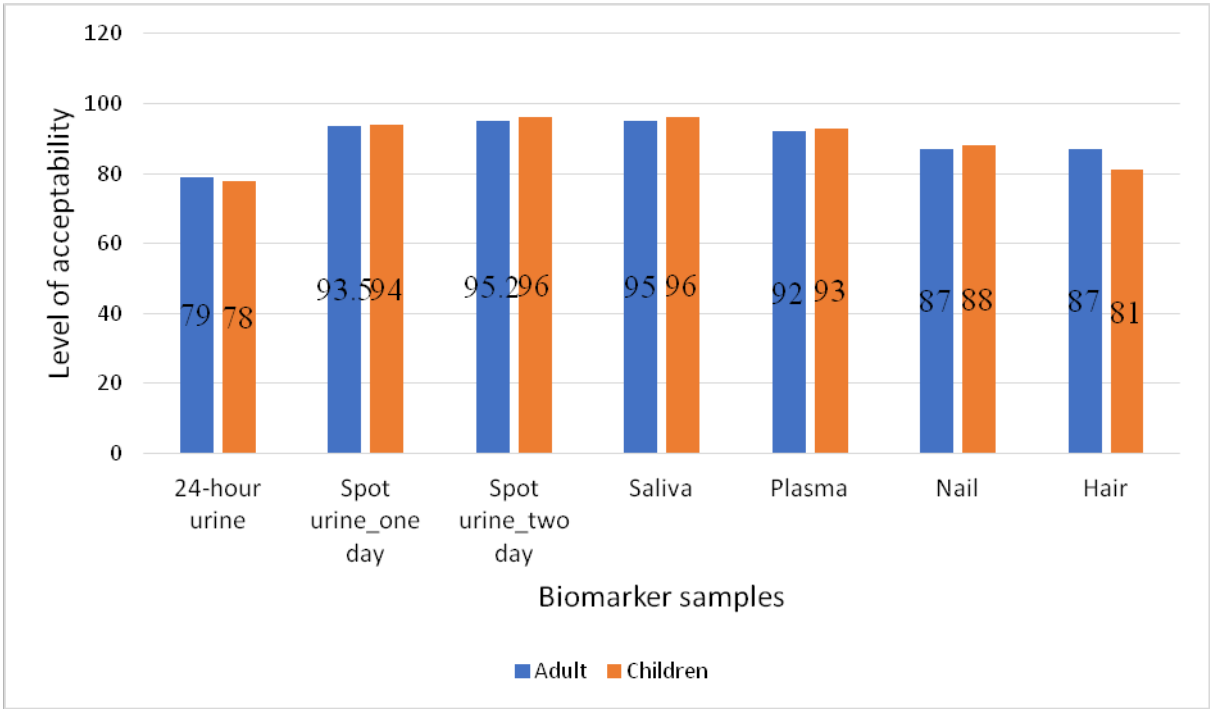

Figure 7.15 Acceptability of biomarker collection in children and adults

7.5 SUMMARY OF THE FINDINGS

Generally, participants were indifferent about the acceptability of the various biological markers, as most of the participants (more than 60%) found all the biomarkers perfectly acceptable. However, there were some differences after considering the ease of collecting the samples. Collection of saliva was ranked the easiest, followed by blood. However, for their children, they found nail collection slightly easier compared to blood whereas they found 24 h urine collection to be the least easy. In as much as they found saliva samples to be the easiest to collect, they had a greater preference for the blood sample for themselves or child, should they be asked to choose among all the biomarkers but, because saliva is very easy to collect, they would rather give it in the future.

7.6 DISCUSSION

7.6.1 Recruitment

Successful recruitment in the present study was associated with two main reasons. Firstly, the researcher engaged with the different stakeholders, including: Commissioner of Ministries (Education and Health), local government representatives, hospital management authorities and community meetings involving the traditional rulers and the elders of the community. The researcher obtained approval from the local government officials, who then provided a representative who introduced the researcher to the traditional rulers. A meeting was then organized with the traditional ruler as well as the community chiefs, where the researcher answered any of their queries. This is usually the method adopted in Africa due to well-established respect for elders/rulers (Molyneux *et al*., 2004; Marshall *et al*. 2007); the people respect the traditional rulers and if anything goes wrong in the communities they are the first point of call.

Secondly, the researcher engaged the public through several meetings to explain the study and answer any enquiries. During the community meeting with the study participants, the researcher discussed the reason for the conduct of the study and that the research was voluntary. They were also informed that the information provided was confidential and strictly for research purposes, and that individuals would not be given the results but a copy of the study would be given to the Ministry of Health for proper consultation (see details in Chapter 8). Participants were very supportive of the fluoride research study and generally had positive attitudes.

7.6.2 Interpretation of study findings

Several studies (Sampaio, 2006; Rugg-Gunn *et al*., 2011; Pessan and Buzalaf, 2011) have investigated the usefulness of the various biological markers of exposure to fluoride but less known is the public view regarding the type of biomarker(s) sample acceptable to donate and under what circumstances the participants would be willing to provide them. A focus group in the UK (Lewis *et al*. 2013) reported several reasons for a strong willingness to donate human biological samples at biomedical facilities, including: 1) it is a good way of reciprocating for medical treatments: where participants who took part in a research are not charged for medical treatment, 2) it is viewed as an important way of developing drugs and treatments, 3) residual tissue which participants did not have any strong emotional ties to would otherwise go to waste and 4) for personal benefit where participants themselves or a

family member was affected by illness or disease. Other UK studies have shown that the public is generally willing to donate tissues and waste material for biomedical research but is more cautious regarding the donation of eyes, brains, lungs and bones (Goodson and Vernon, 2004; Morrel *et al*., 2011). Nordfalk *et al*. (2016) in a telephone survey (N=1195) and 33 qualitative interviews conducted in Demark showed a clear majority were positive or very positive towards organ or tissue donation and this was attributed to self-determination; a strong positive attitude not influenced by monetary benefits. In a study conducted in China, a good number of the participants (75%) were positive about the donation of organs whereas 23% were opposed to the donation (Weiye *et al*., 2017). In a cross-sectional study in Egypt, about 65% reported a positive attitude towards organ donation. Consequently, there might be possible differences in acceptability based on religion (Nordfalk *et al*., 2016), culture (Weiye *et al*., 2011), experiences (Al-Jumah and Abolfotoub, 2011) etc. A study conducted among Taiwanese midlife men and women to examine the potential differences of culture, attitude, social networks, socio demographic factors and religion in a person's quality of life showed that culture involves multiple aspects and interacts with attitudes, social networks and individual factors to influence a person's quality of life (Fu *et al*. 2007). Also, a Japanese study on people's attitude showed culture and previous experience had positive effects on people's attitudes (Bartneck *et al*., 2006). The present study revealed positive attitudes towards the donation of samples, both tissues and fluids. This might be associated with a participant's understanding that collection of these samples enabled the clinicians to know the exact diseases present in the tissues or fluids. Another reason could be that they believe the samples would assist the clinician to study them appropriately.

Interestingly, the study revealed that the differences in their preferences for the individual biological markers was not wide, indicating that participants were indifferent about the collection of any of the biological markers identified in the questionnaire for monitoring fluoride exposure (Figure 7.15). The researcher found from informal discussion with the participants the belief that some diseases might not be present in the blood but in urine, saliva, hair and nails. Also, due to personal benefit prospect from the new treatment resulting from the findings which might still take years. There is profound interest that someday the research will alleviate their symptom (Binka *et al*., 2007). Dasgupta *et al*. (2013) attributed similar findings among an Indian population to altruism; participants were willing to help develop therapies for helping future generations of people with diseases like their own as

well as helping their children and grand-children, which could be due to their present or experience of medical problems and how these problems affected their quality of life. Chatio *et al*. (2016) also showed that participants believed that research had led to the elimination of many diseases such as measles and convulsion in a study conducted among parents whose children were enrolled in a clinical trial in the Kassena-Nankana districts, Ghana between 2000 - 2003.

In the present study, there were slight variations in perceived acceptability between the different biological markers which might still inform us of the biomarker(s) that participants find more acceptable. Participants would prefer to provide saliva for themselves and their children compared to the other biomarkers. This might be associated with the fact that saliva collection is non-invasive, convenient and relatively quick. Interestingly, about 92% of the participants preferred collection of blood whereas 24-hour urine was the least preferred among the biomarkers. The high percentage of participants who preferred to provide blood, which requires an invasive procedure compared to 24-hour urine, might be associated with the prevalence of malaria in the region. Nigeria has approximately 51 million cases of malaria and 207,000 deaths are reported annually, representing 30% of the total malaria burden in Africa, with 97% of Nigeria's total population at risk of infection (WHO, 2014). Due to this risk, people are continually being tested for the infection by blood collection. Participants believed that the conduct of tests influenced the incidence and effect of these diseases. A sample might be acceptable but not practical to collect and this could be associated with, for example: 1) job/daily activity, 2) equipment of collection, 3) convenience of the subject etc. In the present study, there was a drop in the level of acceptability of participants when asked about 24-h urine and this might be associated with the fact that most of the participants were farmers and it might have been difficult to carry the collection container around the farm or to the market when they took their produce to sell. The study revealed that participants also found collection of nails and hair more acceptable compared to 24-hour urine samples. This might be due to the convenience of collecting the samples, although interestingly they preferred blood collection to hair and nail samples. This latter opinion might be associated with the fact that participants were not familiar with the use of hair and nail samples in clinical studies or lack of past-experience related to the use of these samples in hospitals. An informal interview with the participants found that they had never heard of the use of hair and nails in clinical studies and they feared that the samples could be used for ritual purposes. Boahen *et al*. (2013) on community

perception and beliefs about blood collection among community members in the Kintampo district of Ghana showed that participants were afraid the blood sample could be used for ritual purpose except a thorough explanation is provided by the researcher prior to collection of the sample.

Similarly, when asked which sample they find easy to provide among the biomarkers, most participants find the collection of saliva easy, followed by nail and blood but they found the collection of 24-hour urine difficult compared the other biomarkers. The participants were then presented with a scenario in which they were asked to imagine that they were in a hospital waiting room awaiting diagnosis for dental diseases (dental caries and dental fluorosis) and asked which of the samples among urine, saliva, blood, hair, and nail would they like to provide for the medical result. Despite finding saliva more acceptable and easy to collect, the participants preferred to provide blood when asked which they most preferred to provide among all the biomarkers. The fact that they are used to the provision of blood in hospitals since it is often used for most clinical investigation for certain diseases as earlier discussed. In this context, it is interesting to note that the attitude of the Saudi public is favourable towards organ donation and tissue bio-banking due to previous participation in health-related research (Al-Jamah and Abolfotoubi, 2011).

The results were similar with the children when the parents were asked about the collection of samples from their children which is no doubt due to the influence of parents on the children (Figure 7.15). A similar result was reported by Lewis *et al*. (2013) on the willingness to donate human bio-samples for biomedical research among twelve focus groups living in six different geographic locations across the UK. Most respondents were either or probably willing to donate urine (89%), saliva (89%) and blood (81%).

In the present study, a comparison between the numbers of each sample provided by children and adults and the number of participants who expressed a willingness to provide samples, showed a strong relationship between the participants' perception about the biomarkers and the samples they provided. All the participants who had previously said they would be willing to provide samples, provided blood samples as well as saliva whereas 95% provided 24-h urine sample. The least provided samples were hair and nail samples (see Chapter 8).

7.6.3 Validity and trustworthiness

The findings of this study rely on the information provided by the participants on the questionnaire and analysed by the researcher. Validity in research is the degree to which the inferences, conclusions and propositions drawn about the different parts of the study are true and this could be through external (sample size, sample characteristics, sampling method and study settings) or internal validity (Lawrence 2012). The qualitative survey in the current study involved a total sample size of 120 participants (60 parents who also completed a copy of the questionnaire on behalf of their child) and this was considered sufficient to enhance the external validity of the results. The sample size calculation was discussed in Chapter six. A major limitation of the present study is that the research was conducted in a rural setting among black ethnic majority; hence, the results need to be interpreted with caution.

7.6.4 Rationale of the selected assessment methodology

In the present qualitative study, the paper questionnaire was administered to collect information on how participants perceived the biomarkers of exposure to fluoride. This was associated with the practicality of data collection including the fact that the education level of the participants in the study location was low and they might not have been able to complete a web-based questionnaire, having no access to computers and lack of internet access; affordability of smartphones which seemed not to be popular among the participants.

The strength of the present method was that the response rate was 100%: all participants that consented to the study completed the questionnaire. It was easy for the researcher to ensure compliance and that all aspects of the questionnaire were completed. There is a specific limitation to this approach as there are chances that participants might feel that they were being judged and thereby give the answer the researcher wanted to hear which might have led to a biased result and consequently limited the interpretation of the findings.

7.6.5 Conclusions

Generally, participants had a positive attitude toward provision of all the biological markers of exposure to fluoride. It is therefore unlikely that they were going to reject the use of any of the biomarkers. However, they still gave more preference to the use of saliva and blood and least preference for 24-hour urine due to the difficulty in the collection and how it might affect their livelihood. Participants seemed to be unaware of the use of nail and hair samples

for clinical diagnosis as well as having misconceptions about the use of such samples for ritual purposes. Nevertheless, they might provide them when it relates to their health benefit. Based on perceived acceptability of biomarkers of exposure to fluoride, it can be interred from the present study that saliva is the most preferred biomarker, followed by blood. Previous experience, familiarity and daily activity may well be important predictors of attitudes towards the choice of biological markers

CHAPTER 8: STUDY TWO - ASSESSMENT OF RELIABILITY OF BIOLOGICAL MARKERS OF EXPOSURE TO FLUORIDE IN NIGERIA (QUANTITATIVE STUDY)

8.1 INTRODUCTION

This chapter presents the results of the quantitative study and is itemised separately under the following headings: F intake and excretion by F water area, F biomarkers, comparison between children and adults, correlation between F exposure and F biomarkers. The chapter concludes with the effects of age and fluoride water area on biomarkers.

8.2 RECRUITMENT

Recruitment took place between April and July 2016. In total, 64 study packs were distributed in the high- and low-fluoride water areas targeting parents aged ≥20 and their children aged 4-5 years old. In total, 62 parents agreed to take part in the study: 32 parents and their children from the low-fluoride water area and 30 parents and their children from the high-fluoride water area. One participant (and child) withdrew consent in the high fluoride water area after samples were provided following family issues surrounding participation.

The number of recruited participants, as well as the number of participants who completed different aspects of the study, are presented in Table 8.1. Of the biological markers, nail samples (fingernails and toenails) were provided the least.

Table 8.1: Number of participants recruited, and who took part and completed different aspects

Number of Participants:	Low fluoride Area		High Fluoride Area		Total
	Children	Adults	Children	Adults	
Recruited	32	32	30	30	124
Provided consent and took part in the study	31	31	29	29	120
Provided samples of:					
24-h urine	29	30	29	28	116
Saliva	31	31	29	29	120
Fingernails	25	27	25	27	104
Toenails	27	26	27	27	107
Hair	27	29	25	28	109
Blood	31	31	29	29	120

- **Gender**

Table 8.2 shows the number of males and females from both areas who were recruited and participated in the study. More females participated in the study than males. In total, 55.6% (n = 65) of the 120 participants who took part in the study were female.

Table 8.2 Number of participants by gender

Age group	Low fluoride area			High fluoride area			All
	Male	Female	Both genders	Male	Female	Both genders	
Adult	13	18	31	11	18	29	60
Child	13	18	31	18	11	29	60
All	26	36	62	29	29	58	120

8.3 VALIDATION CHECKS

8.3.1 Fluoride analysis

Before full sample analyses were done, repeatability checks were undertaken on the fluoride analytical methods used. Table 8.3 shows the mean difference in the fluoride concentration between test and re-test analyses for all types of sample: urine, blood, saliva, fingernail, toenail, hair and water. The mean difference ranged from 0.006 µg/ml for urine to 0.021 µg/g for toenail samples. There were also no statistically significant differences in the means between test and retest for all variables measured.

Table 8.3 Results for reliability of fluoride analytical method for a minimum of 10% of all samples

Sample type	No of samples	Fluoride concentration (µg/g)		Mean difference (95% CI)	Percentage of the measured values (%)*
		Analysis	Re-analysis		
Urine	12	3.198	3.204	-0.006 (-2.782, +2.769)	0.19
Plasma	12	0.083	0.089	-0.007 (-0.064, +0.050)	8.14
Saliva	12	0.098	0.106	-0.009 (-0.216, +0.199)	8.82
Fingernail	12	3.054	3.070	-0.017 (-2.291, +2.256)	0.56
Toenail	12	7.462	7.441	+0.021 (-6.785, +6.827)	0.28
Hair	12	2.647	2.632	+0.014 (-2.970, +2.998)	0.53
Water	12	2.021	2.034	-0.013 (-1.832, +1.805)	0.64

*Percentage of the measured values= (mean difference/mean measured value) x 100%.

8.3.2 Validation of urine samples (Completeness of 24-h urine)

To avoid drawing incorrect results and conclusions, where participants did not collect their urine sample for 24-h or make up the sample with water to ensure completeness or when sample is collected above 24-h, completeness of 24-h urine was verified. In this study, urinary flow rate was used as a marker to validate the completeness of the 24-h urine sample provided by the participants. According to the WHO recommendation (WHO, 1999), a urinary flow rate (UFR) of <5 ml/h for young children (< 6 years) and <9 ml/h for older children (≥ 6 years) and adults should be considered as invalid. Based on these criteria, one child with a URF of 4.0 ml/h and two adults with URFs of 7.3 and 6.0 ml/h from the low fluoride water area as well as one adult with a UFR of 4.1 ml/h from the high fluoride area were excluded from the data analysis (Table 8.4)

Table 8.4 Number of valid and invalid 24 h urine samples collected from low and high fluoride areas.

Number of:	Low fluoride Area (0.04 mgF/l)		High Fluoride Area (3.05 mgF/l)	
	Children	Adult	Children	Adult
Collected 24-h urine samples	29	30	29	28
Invalid 24-h urine samples	1	2	0	1
Valid 24-h urine samples	28	28	29	27

8.4 FLUORIDE CONCENTRATION OF HOME DRINKING WATER, DRINKS AND FOOD

8.4.1 Fluoride concentration of drinking water supply

The mean fluoride concentration of drinking water for the low- and high- fluoride water areas was 0.04 (0.02) and 3.05 (1.10) mg/l, respectively.

8.4.2 Fluoride concentration of consumed food and drinks recorded in the food frequency questionnaire (FFQ)

Table 8.5 shows the fluoride concentration (µg/ml or µg/g) of food and drinks commonly consumed by the parents and their children extracted from the questionnaire. There is a wide range of fluoride concentration in foods and drinks in the different fluoride areas. In the low fluoride area, the highest fluoride concentration was found in tea bags prepared with milk (2.780 µg/ml) followed by the most consumed local soup, Kuka soup, rice and fish with fluoride concentrations 2.255 µg/ml, 1.597 µg/ml and 1.449 µg/ml respectively, while the lowest fluoride concentration was found in maize when prepared as paste (0.040 µg/ml) to be eaten with the soup or drunk as liquid (0.031 µg/ml). In the high fluoride area, the highest fluoride concentration is found in the local gin fermented from maize using the local water (5.176 µg/ml) followed by the commonly consumed "moringa soup" (2.783 µg/ml) and maize drink (2.429 µg/ml) and lowest fluoride concentration was found in spinach soup (0.117 µg/ml). Other food and drinks that were recorded in the FFQ where the fluoride concentration was not measured in the field were obtained from the UK fluoride database (Zohoori and Maguire, 2015) and US fluoride database (Cutrufelli *et al*., 2004) (see Appendix 22) as well as from other studies conducted in Nigeria (Ibiyemi *et al*., 2016).

Table 8.5A Fluoride concentration of drink items analysed in this project

Drink item	Source*	Mean concentration (µg/ml)	
		Low fluoride area	High fluoride area
Lipton tea bag (with milk)	Prepared	2.780	n/a
Chocolate drink (with milk)	Prepared	0.066	n/a
Coke	Purchased	0.345	n/a
Maize drink (Kunu)	Prepared	0.031	2.429
Local alcohol drink (Burukutu)	Purchased	n/a	5.176

** In the low fluoride area, food and drinks were prepared with water containing 0.04 mg/l fluoride. In the high fluoride area, food and drinks were prepared with water containing 3.05 mg/l fluoride.*

n/a, drinks not consumed

Table 8.5B Fluoride concentration of food items analysed in this project

Food item	Source*	Mean concentration (µg/g)	
		Low fluoride area	High fluoride area
Bean (mixed with rice)	Prepared	0.094	0.870
Beans (mixed with potatoes)	Prepared	0.152	n/a
Beans processed	Prepared	0.092	1.045
Rice	Prepared	1.597	n/a
Acha (i.e. hungry man rice)	Prepared	0.053	n/a
Maize	Prepared	0.040	0.977
Okro soup	Prepared	0.631	n/a
Kuka soup (local vegetable)	Prepared	2.255	n/a
Spinach soup	Prepared	n/a	0.117

Food item	Source*	Mean concentration (μg/g)	
		Low fluoride area	High fluoride area
Moringa soup	Prepared	n/a	2.783
Potatoes	Prepared	0.039	n/a
Fish	Prepared	1.449	n/a
Bread	Purchased	0.145	0.450
Cassava	Prepared	n/a	0.228
Guinea corn	Prepared	n/a	1.189
Meat (dog)	Purchased	n/a	0.312
Meat (Pork)	Purchased	n/a	0.998

** In the low fluoride area, food and drinks were prepared with water containing 0.04 mg/l fluoride. In the high fluoride area, food and drinks were prepared with water containing 3.05 mg/l fluoride*

n/a, food not identified in FFQ.

8.5 ANTHROPOMETRIC CHARACTERISTICS OF PARTICIPANTS

The anthropometric characteristics of the 120 children and adults, who took part in the study, are presented in Tables 8.6 and 8.7, respectively, by area. There was no significant difference in the data for the two fluoride water areas.

Table 8.6 Anthropometric characteristics of children (n=60) in the study

Anthropometric characteristics	Mean (SD)		Mean Differences (95% CI)	P value
	Low Fluoride Area (n=31)	High Fluoride Area (n=29)		
Age (years)	4.4 (0.7)	4.4 (0.7)	-0.00 (-0.37, 0.36)	0.982
Weight (kg)	16.3 (2.6)	15.7 (5.0)	-0.66 (-2.66, 1.33)	0.510
Height (cm)	100.8 (7.9)	100.9 (11.3)	0.04 (-4.89, 4.97)	0.988
BMI (kg/m^2)	16.2 (2.8)	15.2 (2.2)	-1.01 (-2.29, 0.26)	0.118

Table 8.7 Anthropometric characteristics of adults (n=60) in the study

Anthropometric characteristics	Mean (SD)		Mean Differences (95% CI)	P value
	Low Fluoride Area (n=31)	High Fluoride Area (n=29)		
Age (years)	33.1 (5.4)	34.6 (12.2)	1.47 (-3.40, 6.35)	0.534
Weight (kg)	67.5 (8.2)	64.8 (11.6)	-2.73 (-7.80, 2.34)	0.285
Height (cm)	164.7 (163.2)	163.2 (9.5)	-1.54 (-6.42, 3.33)	0.530
BMI (kg/m^2)	25.0 (3.6)	24.4 (4.4)	-0.62 (-2.67, 1.44)	0.549

8.6 ORAL HYGIENE DATA

Table 8.8 shows the oral hygiene information of children who participated in the study by area. All the participants (100%) from the low fluoride area claimed to brush their teeth; whereas the corresponding figure was 80% in the high fluoride area. Most of the participants brushed their teeth once per day in both fluoride areas (96.9% and 95.8% in low- and high fluoride water areas respectively). Close-up was the most used brand of toothpaste in both fluoride areas (84.4% and 76.7% in the low- and high fluoride area respectively). Among the children, 87.5% and 95.8% claimed to use toothpaste labelled by the manufacturer as containing a fluoride concentration of 1450 ppm in the low- and high fluoride water areas respectively.

Table 8.8 Information on tooth brushing habits, type and fluoride concentration of toothpaste used by children (n=60)

	% of children by fluoride area		
	Low Fluoride Area (n=31)	High Fluoride Area (n=29)	All (n=60)
Do you brush your teeth?			
Yes	100	80	90.3
No	0	20	9.7
No. of brushings/day			
Once	96.9	95.8	96.4
Twice	3.1	4.2	3.6
Type of toothpaste used			
Close-up	84.4	76.7	77.4
Herbal Close-up	3.1	0	1.6
Macleans	3.1	20	1.6
Oral B	9.4	3.3	6.5
Fluoride concentration of toothpaste used (ppm)			
1100	12.5	4.2	8.9
1450	87.5	95.8	91.1
Who put toothpaste on tooth brush?			
Parent	78.1	100	79.1
Self	21.9	0	11.3
Who brushes the teeth?			
Parent	43.7	96.7	59.7
Self	56.3	3.3	30.6

Table 8.9 shows the oral hygiene information of adults who participated in the study by area. The percentage of adults who brushed their teeth was the same as that of the children in both fluoride areas. Most of the participants in the high fluoride area claimed they brushed their teeth once a day (91%, n=30) while 71% of participants brushed once in the low fluoride

area and the remaining few in both fluoride areas brushed twice per day. Among the adults, 75% and 76.7% used Close-up toothpaste for brushing their teeth in the low and high fluoride area respectively while the others used brands like Herbal Close-up, Macleans and Oral B, as Close-up toothpaste is the cheapest and most readily available among the brands. All the toothpastes used by the participants contained fluoride in the form of sodium fluoride (NaF). With regards to the amount of fluoride in the toothpaste used as labelled by the manufacturer, most of the participants used toothpaste labelled 1450 ppm in the low and high fluoride area for brushing their teeth (80.6% and 95.8% in low and high fluoride area respectively) while the remaining participants used toothpaste containing 1100 ppm fluoride.

Table 8.9 Information on tooth brushing habits, type and fluoride concentration of toothpaste used by adults (n=60)

	% of adult by fluoride area		
	LFA (n=31)	HFA (n=29)	All (n=60)
Do you brush your teeth?			
Yes	100	80	90.3
No	0	20	9.7
No. of brushings/day			
Once	71.0	91.7	78.6
Twice	29.0	8.3	21.4
Type of toothpaste used			
Close-up	75.0	76.7	74.2
Herbal Close-up	3.1	0	1.6
Macleans	9.4	20	6.4
Oral B	9.4	3.3	6.5
Fluoride concentration of toothpaste used (ppm)			
1100	19.4	4.2	12.8
1450	80.6	95.8	87.3

8.7 FLUORIDE INTAKE FROM DIET

8.7.1 Children

8.7.1.1 Weight of consumed foods, drinks and water

Table 8.10 shows the mean (SD) weights of foods, drinks and water consumed by children living in the low and high fluoride areas. Although the mean weight of food and drinks consumed by children living in the high fluoride area was higher than that of the low fluoride area, the difference was not statistically significant.

Table 8.10 Comparison of the mean estimated weight (g) of food, drinks and water consumed by children (n=60) in high and low fluoride areas

Source	Weight (g/day) Mean (SD)		Difference in weight (g/day) Mean (95% CI)	P value
	Low fluoride	High fluoride		
Total drinks	607 (224)	707 (380)	-99.7 (+63.36, -262.7)	0.224
Water	*420 (151)*	*457 (200)*	*-37.5 (+54.4, -129.4)*	*0.417*
Other drinks	*188 (124)*	*250 (295)*	*-56.1 (+63.4, -175.7)*	*0.347*
Foods	1525 (432)	1783 (696)	--258.6 (+43.4, -560.5)	0.091
Total	2132 (568)	2490 (877)	-202.9 (-636.8, +231.0)	0.352

8.7.1.2 Daily fluoride intake from individual food and drink groups by fluoride area

Table 8.11a and 8.11b shows the fluoride intake from each food and drink group by fluoride area. In the low fluoride water area, tea contributed 25% to the overall dietary fluoride intake. Of all that was consumed by the children, milk processed from cows and mixed with a local cereal contributed the highest percentage (34%). However, in the high fluoride area, borehole water and alcoholic drinks (prepared with the borehole water) were the highest contributors to the overall dietary fluoride intake (29 and 31% respectively). The contribution from tea bags was the highest among the drink group in the low fluoride area (273.51 ml/day), whereas contributions from well water (2.34 ml/day), herbal tea bags (2.31 ml/day) and fruit drinks (2.76 ml/day) were low. Children in the low fluoride area consumed

no alcohol. In the high fluoride area, there was a relatively high contribution from the different sources of drinking water that the children drank from, including well water (604.34 ml/day), borehole water (947.80 ml/day) and tap water (government supplied) (634.30 ml/day). However, the highest contribution was from locally brewed alcoholic drinks with an average daily intake of 2233.83 ml/day.

Among the food groups, the highest contribution in the low fluoride area was from rice (20%) However, in the high fluoride area, there were no significant contribution to the overall dietary fluoride among the food groups.

Table 8.11A Fluoride intake of drink groups by fluoride area

Drink groups	Low fluoride area		High fluoride area	
	% contribution to TDDFI	Mean (SD) intake (ml/day)	% contribution to TDDFI	Mean (SD) intake (ml/day)
Tap water	2.2	20.08 (6.78)	0	0
Well water	0.3	2.34 (1.79)	12.3	604.34 (388.69)
Borehole water	1.2	7.94 (4.29)	29.3	947.80 (582.93)
Sachet water	1.9	17.37 (11.77)	0.8	21.05 (20.89)
Bottle water	2.0	17.48 (10.63)	0.4	15.00
Tap water	4.4	42.17 (38.92)	17.5	634.30 (489.82)
Tea bags	25.7	273.51 (186.56)	6.4	194.09 (195.69)
Herbal tea bags	0.2	2.31 (1.29)	0	0
Coffee	0.3	4.11 (1.05)	1.0	28.46
Chocolate drink	0.7	4.99 (4.29)	0.8	25.52 (15.84)
Alcoholic drink	0	0	31.9	2233.83 (1905.56)
Soft drink	1.9	16.60 (12.86)	0.7	16.61 (13.41)
Fruit drink	0.3	2.76 (3.21)	0.2	8.25 (13.89)

Drink groups	Low fluoride area		High fluoride area	
	% contribution to TDDFI	Mean (SD) intake (ml/day)	% contribution to TDDFI	Mean (SD) intake (ml/day)
Processed milk	2.0	21.08 (12.50)	0.5	15.79 (9.12)
Other milk	34.2	550.85 (590.14)	15.9	777.14 (595.46)

TDDFI, Total Daily Dietary Fluoride Intake (food and drinks)

Table 8.11B Fluoride intake of food groups by fluoride area

Food groups	Low fluoride area		High fluoride area	
	% contribution to TDDFI	Mean (SD) intake (µg/day)	% contribution to TDDFI	Mean (SD) intake (µg/day)
Egg	1.1	9.30 (7.97)	0.3	8.10 (10.77)
Cheese	0.1	0.61 (0.57)	0.0	0.40 (0.30)
Rice	20.2	165.97 (103.81)	5.9	181.85 (144.49)
Maize (paste)	1.1	6.47 (5.66)	7.7	225.06 (91.48)
Maize (Akamu)	1.3	7.80 (5.10)	1.0	29.94 (16.27)
Pasta	3.8	26.60 (16.90)	7.4	237.60 (229.10)
Indomine (pasta)	0.8	6.40 (3.77)	0.3	20.65
Bread	0.1	0.96 (0.87)	0.0	0.93 (1.06)
Breakfast cereals	0.2	1.19 (0.28)	0.1	1.99
Yam tuber	2.2	19.61 (14.80)	0.9	24.12 (23.74)
Potato	1.1	7.30 (3.79)	1.3	49.39 (29.82)
Cassava	2.7	22.07 (19.21)	0.9	28.55 (19.83)
Plantain	0.0	0.07 (0.04)	0.0	0.14 (0.13)
Meat	0.4	3.03 (1.97)	0.2	5.74 (6.41)

Food groups	Low fluoride area		High fluoride area	
	% contribution to TDDFI	Mean (SD) intake (µg/day)	% contribution to TDDFI	Mean (SD) intake (µg/day)
Dried meat	7.9	76.00 (44.80)	5.0	272.73 (342.19)
Fish	7.9	62.73 (26.73)	2.7	73.13 (52.92)
Canned fish	2.4	19.76 (21.48)	0.6	37.64
Game meat	1.0	12.28 (14.50)	0.5	17.47 (14.97)
Meat product	0.1	0.51 (0.61)	0.0	0.16
Beans	1.0	7.02 (4.17)	2.2	65.72 (40.13)
Processed beans	2.0	16.59 (12.09)	5.6	177.34 (151.37)
Soup	11.4	91.50 (63.36)	4.4	126.71 (79.64)
Mango	0.2	1.77 (1.09)	0.1	4.05 (3.24)
Pear	0.1	1.23 (0.74)	0.0	1.16
Pineapple	0.0	0.51 (0.38)	0.0	0.69
Water melon	0.0	0.32 (0.27)	0.0	0.49 (0.46)
Oranges	0.2	1.20 (0.70)	0.1	2.34 (2.15)
Cashew nuts fruit	0.3	2.17 (1.42)	0.1	6.50
Acha (Hungry man rice)	0.3	1.66 (1.59)	0	0

TDDFI, Total Daily Dietary Fluoride Intake (food and drinks)

8.7.1.3 Total Daily dietary fluoride intake (TDDFI) from foods and drinks groups

Table 8.12 shows TDDFI from the two major dietary sources per day (mg/d) and on a body weight basis (mg/kgbw/d) for each fluoride water area. The fluoride contribution from food (0.735 mg/d) was higher than that from all drinks (including water) (0.265 mg/d) in the low fluoride area, as most of the food consumed was not produced in the area (Halo effect); while in high fluoride area, the contribution from all drinks (including water) (2.201 mg/d) was higher than that from food (1.412 mg/d. The highest contribution to fluoride intake in

the low fluoride area is from foods (73.7%) whereas drinks (including water) contributed more to the fluoride intake in the high fluoride area (60.9%) among the children. However, drinks contributed 26.3% in the low fluoride area while food contributed 39.1% in high fluoride area. The mean DDFI from drinks (excluding water) (0.226 mg/d) was higher than the contribution from water (0.039 mg/d) in the low fluoride area even though the children drank more water. The mean DDFI values for all major dietary sources were statistically significantly higher in the high fluoride area than in the low fluoride area: water ($p<0.001$), drinks excluding water ($p<0.003$) and food ($p<0.001$).

There was a significant difference in the contribution to the TDDFI from drinks ($p < 0.001$) between the low- and high fluoride water areas as well as in the respective contributions from food ($p = 0.001$). There was also a significant difference ($p < 0.001$) between total DDFI in the high fluoride area [3.738 (2.184) mg/d] and that in the low fluoride area [0.985 (0.603) mg/d].

Table 8.12 Comparison of the mean fluoride intakes from the two main dietary sources in the low- and high water fluoride areas.

Source	Fluoride Intake Mean (SD)		Difference in fluoride intake Mean (95% CI)	P value
	Low fluoride	High fluoride		
All drinks (including water)				
mg/d	0.259 (0.209)	2.277 (1.789)	-2.018 (-1.334, -2.703)	<0.001
mg/kgbw/d	0.015 (0.012)	0.166 (0.148)	-0.151 (-0.094, -0.207)	<0.001
Drinking water (mg/d)	*0.036 (0.030)*	*1.184 (0.631)*	*-1.148 (-0.908, -1.388)*	<0.001
Other drinks (mg/d)	*0.223 (0.202)*	*1.093 (1.405)*	*-0.870 (-0.332, -1.409)*	*0.003*
Foods				
mg/d	0.726 (0.536)	1.461 (0.843)	-0.735 (-0.367, -1.103)	0.001
mg/kgbw/d	0.045 (0.035)	0.100 (0.062)	-0.054 (-0.028, -0.081)	<0.001
Total DDFI				
mg/d	0.985 (0.603)	3.738 (2.184)	-2.614 (-3.477, -1.750)	<0.001
mg/kgbw/d	0.060 (0.038)	0.266 (0.183)	-0.205 (-0.134, -0.276)	<0.001

8.7.2 Adults

8.7.2.1 Weight of consumed foods, drinks and water

The mean (SD) weights of foods and drinks consumed by the adults living in the high- and low fluoride water areas are presented in Table 8.13. There were no statistically significant differences in the mean weight of consumed foods, drinks, and water between the two fluoride areas. Although the adults living in the high fluoride area consumed numerically more food and drinks (including water) on average than adults living in the low fluoride area, this difference is not significant. Adults living in the low fluoride area drank more water on average than those living in the high fluoride area but the difference in their consumption is not significant. Table 8.13 also shows that participants living in both areas consumed a similar quantity of food.

Table 8.13 Comparing the mean estimated weight (g) of food, drinks and water consumed in the high- and low fluoride water areas

Source	Weight (g/day) Mean (SD)		Difference in weight (g/day) Mean (95% CI)	P value
	Low fluoride	High fluoride		
Total drinks	1240 (563)	1354 (939)	-113.6 (+290.9, -518.1)	0.575
Water	*829 (326)*	*704 (244)*	*+125.0 (+271.8, -21.8)*	*0.094*
Other drinks	*411 (364)*	*650 (852)*	*-238.6 (+107.3, -584.5)*	*0.171*
Foods	2749 (729)	3036 (1469)	-286.2 (+323.5, -896.0)	0.348
Total	3989 (980)	4389 (1999)	-399.8 (+427.9, -1227.6)	0.335

8.7.2.2 Daily fluoride intake from individual food and drink groups by fluoride area

Table 8.14 shows the fluoride contributions from the foods and drinks groups by fluoride area. The major contribution among the drinks group in the low fluoride area was from tea bags. While in the high fluoride area, the major contributions to the drinks group are alcoholic drinks and drinking water sources. Milk (from cow and soy) was another major contributor to the daily dietary fluoride intake in the low fluoride water area as well as in the high fluoride water area.

Among the food groups in the low fluoride area, certain food types, e.g. rice, maize, pasta and dried meat, contributed markedly to the overall daily intake from food in the high fluoride area. Most of the foods and drinks consumed in the low fluoride area were equally consumed in the high fluoride area except for local alcohol which was only consumed by adults living in the high fluoride area. Considering the overall dietary fluoride intake among adults in the high fluoride area, major contributions were from alcohol (locally brewed with borehole water) (40%) and borehole water consumption (30%). For adults in the low fluoride area, major contribution to the total dietary fluoride intake were from a local drink sourced from cow milk and maize (34%), tea (26%) and rice (22%) (See Table 8.14a). Interestingly, these foods and drinks were not produced locally (Halo effect).

Table 8.14A Fluoride intake from drink groups by fluoride area

Drink groups	Low fluoride area		High fluoride area	
	% contribution to TDDFI	Mean (SD) intake (ml/day)	% contribution to TDDFI	Mean (SD) intake (ml/day)
Tap water	3.8	58.38 (26.97)	0.1	24.5
Well water	0.3	4.12 (2.85)	8.6	822.09 (544.26)
Borehole water	1.1	14.38 (6.11)	29.8	1437.50 (841.35)
Sachet water	1.9	31.18 (24.54)	0.7	30.36 (29.93)
Bottle water	3.0	46.44 (59.35)	0.1	8.04
Tap water	2.7	51.02 (40.50)	14.7	1077.49 (789.50)
Tea bags	26.0	510.01 (452.22)	6.9	378.39 (323.13)
Herbal tea bags	0.3	4.86 (3.24)	0.4	45.70
Coffee	0.3	5.60 (2.37)	0.2	7.11

Drink groups	Low fluoride area		High fluoride area	
	% contribution to TDDFI	Mean (SD) intake (ml/day)	% contribution to TDDFI	Mean (SD) intake (ml/day)
Chocolate drink	0.7	9.76 (10.02)	0.8	43.96 (27.94)
Alcoholic drink	0	0	39.9	5459.49 (5096.06)
Soft drink	2.1	35.91 (32.58)	0.6	26.61 (26.11)
Fruit drink	0.4	4.92 (5.77)	0.1	7.87 (10.50)
Processed milk	1.3	30.40 (25.85)	0.8	46.06 (57.02)
Other milk	34.3	1003.03 (1170.00)	13.4	1504.42 (1636.84)

Table 8.14B Fluoride intake from food groups by fluoride area

Food groups	Low fluoride area		High fluoride area	
	% contribution to TDDFI	Mean (SD) intake (µg/day)	% contribution to TDDFI	Mean (SD) intake (µg/day)
Egg	1.1	17.84 (16.72)	0.2	8.42 (7.71)
Cheese	0.1	1.47 (1.94)	0.0	0.75 (0.69)
Rice	21.6	336.28 (226.55)	5.8	319.93 (217.42)
Maize	1.1	13.97 (8.47)	8.0	426.87 (205.61)
Maize drink	1.2	14.14 (6.49)	0.9	45.35 (23.38)
Pasta	3.2	45.03 (33.78)	6.1	380.03 (334.15)
Indomine (pasta)	0.7	8.95 (6.11)	0.8	42.74 (25.43)
Bread	0.1	1.81 (1.36)	0.0	1.59 (1.69)
Breakfast cereals	0.2	3.89 (3.77)	0.0	1.99
Yam tuber	2.2	40.79 (30.62)	0.7	42.87 (39.80)

Food groups	Low fluoride area		High fluoride area	
	% contribution to TDDFI	Mean (SD) intake (µg/day)	% contribution to TDDFI	Mean (SD) intake (µg/day)
Potato	1.2	15.07 (8.60)	1.3	134.30 (89.47)
Cassava	2.2	28.83 (21.19)	1.0	75.39 (64.32)
Plantain	0.0	0.25 (0.22)	0.0	0.22 (0.24)
Meat	0.2	3.35 (2.09)	0.1	8.50 (9.71)
Dried meat	7.1	135.82 (153.02)	4.4	528.00 (714.14)
Fish	4.9	70.79 (37.88)	2.4	100.43 (71.25)
Canned fish	1.6	22.36 (23.55)	1.1	79.05 (103.43)
Game meat	0.7	14.26 (18.96)	0.3	9.98
Meat product	0.0	0.74 (0.87)	0.0	0.16
Beans	1.1	16.52 (12.94)	2.8	160.39 (146.28)
Processed beans	1.8	26.52 (22.57)	4.2	224.59 (161.09)
Soup	8.9	161.98 (218.06)	3.9	177.07 (132.23)
Mango	0.1	2.31 (1.78)	0.1	6.19 (5.85)
Pear	0.1	1.85 (1.58)	0.0	0.96 (0.27)
Pineapple	0.0	0.82 (1.07)	0.0	0.68
Water melon	0.0	0.39 (0.30)	0.0	0.71 (0.63)
Oranges	0.1	1.74 (1.07)	0.1	4.00 (4.44)
Cashew nuts fruit	0.1	1.55 (0.54)	0.1	12.44 (7.59)
Acha (Hungry man rice)	0.4	6.10 (6.31)	0.1	5.15

8.7.2.3 Total Daily dietary fluoride intake (TDDFI) from foods, drinks and water

Table 8.15 shows the daily dietary fluoride intake from foods, drink and water. There was a statistically significant difference in TDDFI from drinks (including water) and food between the low- and high fluoride areas. The mean TDDFI from food consumed by adults was statistically significantly different ($p < 0.05$) between low fluoride (1.359 mg/d) and high fluoride (2.496 mg/d) areas. Fluoride intake from drinks (excluding water) contributed more to the overall intake from drinks in the low fluoride area, most of which are sourced externally. However, the contribution of foods to the total daily dietary fluoride intake was higher compared to drinks (including water). Conversely, in the high fluoride area, the contribution from drinks (including water) was almost double the contribution from foods and might be attributed to the fluoride concentration in the drinking water. A major contribution to the total dietary intake was from food in both the low- and high fluoride areas. The total daily fluoride intake from the diet when adjusted by weight was 0.029 and 0.116 mg/kgbw/d from the low and high fluoride areas, respectively.

There were significant differences in the fluoride contribution from both drinks ($p < 0.001$) and food ($p = 0.006$) between the low fluoride area and the high fluoride area. The highest contribution to fluoride intake in the low fluoride area was from foods (71.8%), whereas drinks (including water) contributed more to the fluoride intake (65.6%) in the high fluoride area among the adults. The fluoride contribution from drinks in the low fluoride area was 28.2% and the contribution from food in the high fluoride area was 34.4%.

Table 8.15 Comparison of the mean fluoride intake from two main dietary sources (mg/d and mg/kgbw/day) in the low- and high- fluoride water areas.

Source	Intake (g/day) Mean (SD)		Difference in weight (g/day) Mean (95% CI)	P value
	Low fluoride	High fluoride		
All drinks (including water)				
mg/d	0.537 (0.487)	4.933 (4.785)	-4.396 (-2.569, -6.223)	<0.001
mg/kgbw/d	0.008 (0.007)	0.078 (0.075)	-0.070 (-0.042, -0.099)	<0.001
Drinking water (mg/d)	*0.082 (0.058)*	*1.867 (0.903)*	*-1.785 (-1.441, -2.129)*	<0.001
Other drinks (mg/d)	*0.454 (0.472)*	*3.065 (4.396)*	*-2.611 (-0.932, -4.290)*	*0.004*
Foods				
mg/d	1.367 (1.106)	2.582 (1.992)	-1.214 (-0.370, -2.058)	0.006
mg/kgbw/d	0.021 (0.017)	0.041 (0.034)	-0.021 (-0.006, -0.035)	0.005
Total DDFI				
mg/d	1.904 (1.218)	7.515 (5.810)	-5.610 (-3.364, -7.857)	<0.001
mg/kgbw/d	0.029 (0.019)	0.120 (0.095)	-0.091 (-0.055, -0.128)	<0.001

8.8 FLUORIDE INTAKE FROM TOOTHPASTE INGESTION

8.8.1 Children

Table 8.16 shows the amount of toothpaste dispensed and the amount of fluoride ingested per brushing by fluoride area. There was no statistically significant difference in the mean weight of dispensed toothpaste per brushing between the low- and high fluoride areas. There was also no statistically significant difference in the amount of fluoride ingested per brushing and per day. When the amount ingested per brushing and per day was normalised by weight, no statistically significant difference was found between the low- and high fluoride area.

8.8.2 Adults

The amount of toothpaste dispensed and fluoride ingestion by adults in each fluoride area is presented in Table 8.17. There was statistically no significant difference in the amount of toothpaste dispensed on toothbrush between adults in the low- and high fluoride areas. The

mean fluoride ingestion from toothpaste (mg/brushing and mg/day) was not statistically significantly different between the two fluoride areas when analysed using an independent *t* test. When the fluoride ingestion was normalised by body weights (mg/kgbw/brushing and mg/kgbw/day), intake from toothpaste was also not statistically significantly different between the two areas.

Table 8.16 Comparison of mean toothpaste usage and ingestion by children (n=55) in low- and high- fluoride water areas

	Mean (SD) Fluoride area		Mean Difference (95% CI)	P value
	Low (n = 31)	High (n = 24)		
Weight of toothpaste on toothbrush (g)	0.266 (0.167)	0.203 (0.132)	0.063 (-0.018, +0.142)	0.124
Fluoride ingestion				
mg/brushing	0.224 (0.135)	0.171 (0.084)	0.053 (-0.006, +0.112)	0.077
mg/day	0.228 (0.135)	0.191 (0.169)	0.038 (-0.046, +0.123)	0.370
Fluoride ingestion				
mg/kgbw/brushing	0.014 (0.008)	0.012 (0.008)	0.002 (-0.002, +0.006)	0.368
mg/kgbw/day	0.014 (0.008)	0.014 (0.160)	0.000 (-0.007, +0.008)	0.900

Table 8.17 Comparison of the mean toothpaste usage and ingestion by adults (n=55) in low- and high fluoride areas

	Mean (SD)		Mean Difference (95% CI)	P value
	Low fluoride area n=31	High fluoride area n=24		
Weight of toothpaste on toothbrush (g)	0.368 (0.253)	0.468 (0.266)	-0.082 (-0.224, +0.059)	0.250
Fluoride ingestion				
mg/brushing	0.328 (0.228)	0.405 (0.229)	-0.076 (-0.201, +0.049)	0.228
mg/day	0.430 (0.332)	0.452 (0.310)	-0.022 (-0.197, +0.152)	0.800
Fluoride ingestion				
mg/kg bw/brushing	0.005 (0.003)	0.006 (0.005)	-0.001 (-0.003, +0.000)	0.156
mg/kgbw/day	0.006 (0.005)	0.007 (0.005)	-0.000 (-0.003, +0.001)	0.699

8.9 TOTAL DAILY FLUORIDE INTAKE (TOOTHPASTE AND DIET)

8.9.1 Children

Overall, total daily fluoride intake (mg/d and mg/kgbw/d) from all sources (diet and toothpaste) among children in the high fluoride area was statistically significantly higher than in the low fluoride area. Total daily fluoride intake (diet and toothpaste) was 1.214 (0.575) mg/d and 0.075 (0.036) mg/kgbw/d in the low fluoride area and 3.896 (2.147) mg/d and 0.277 (0.184) mg/kgbw/d in the high fluoride area.

8.9.2 Adults

Overall, the mean (SD) TDFI from all sources (diet and toothpaste) in the high fluoride area was significantly higher than that in the low fluoride area. On a body weight basis, TDFI (diet and toothpaste) was statistically significantly ($p < 0.001$) higher in the high fluoride area (0.125 (0.093) mg/kgbw/d) than in the low fluoride area (0.036 (0.020) mg/kgbw/d). Total daily fluoride intake in mg/d was 2.321 (1.278) and 7.889 (5.721) in low- and high fluoride areas, respectively.

8.10 Percentage of participants with optimal fluoride intake

8.10.1 Children

Figure 8.1 shows the percentage of children within the suggested optimal range of fluoride intake. In the high fluoride area, the TDFI of only 3.3% of the children was within the suggested optimal range (0.05 – 0.07 mg/kgbw/d), whereas the TDFI was higher than the threshold of the tolerable upper intake level (0.1 mg/kgbw/d) for 80% of the children. However, in the low fluoride area, 28.1% of the children received optimal fluoride intake while 12.5% exceeded the tolerable upper intake level.

8.10.2 Adults

The percentage of adults with suggested optimal and tolerable upper intake levels for fluoride intake are presented in Figure 8.2. In the high fluoride area, 13.3% of the adults had a TDFI within the suggested optimal range for fluoride intake (0.05 – 0.07 mg/kgbw/d), whereas for 50% the TDFI was higher than the threshold of the tolerable upper intake level (0.1 mg/kgbw/d). Conversely, only 9.4% of the adults received optimal fluoride intake in the low fluoride area and no adult exceeded the tolerable upper intake level for fluoride intake. A total of 84.4% of the adults living in the low fluoride area received suboptimal fluoride intake.

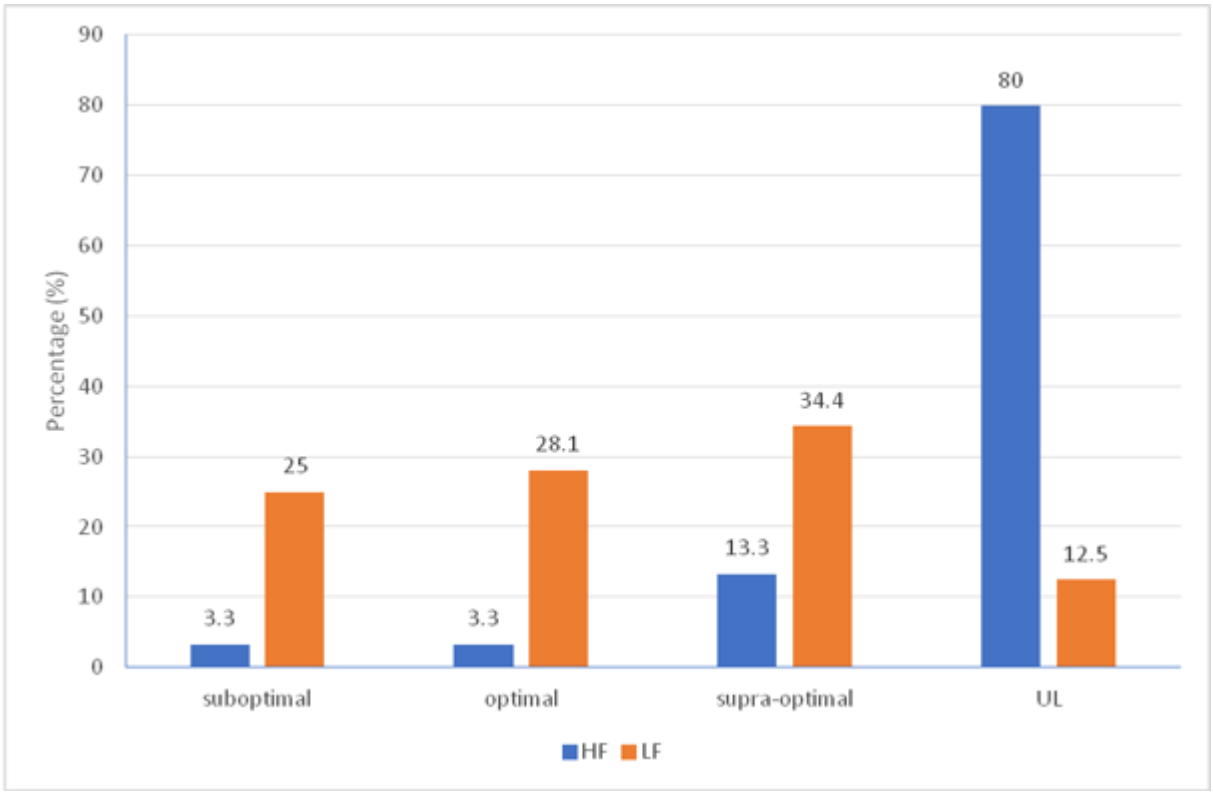

Figure 8.1 Percentage of children with suggested suboptimal (< 0.05 mg/kgbw/d), optimal (0.05 – 0.07 mg/kgbw/d), supra-optimal (> 0.07 to < 0.1 mg/kgbw/d) and tolerable upper intake level (UL) of fluoride intake (≥ 0.1mg/kgbw/d) in the low- and high fluoride areas.

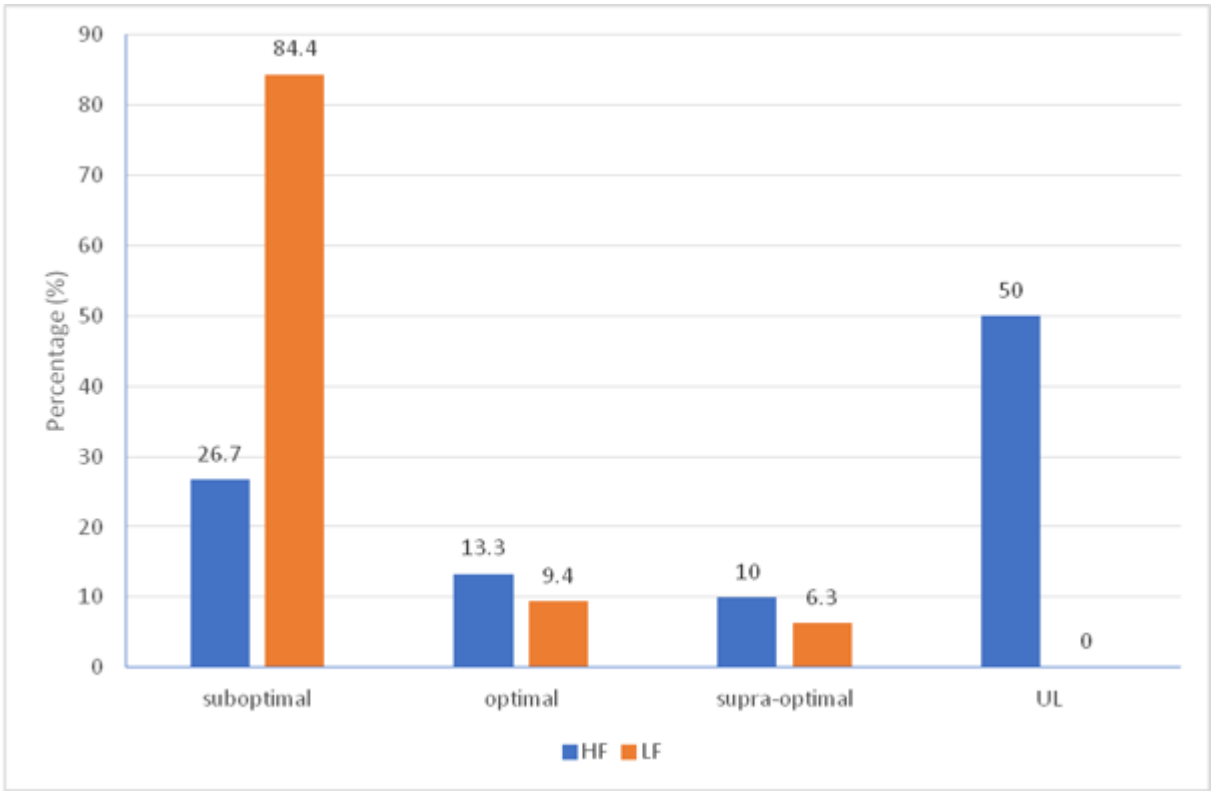

Figure 8.2 Percentage of adults with suggested suboptimal (< 0.05 mg/kgbw/d), optimal (0.05 – 0.07 mg/kgbw/d), supra-optimal (> 0.07 to < 0.1 mg/kgbw/d) and tolerable upper intake level (UL) of fluoride intake (≥ 0.1mg/kgbw/d) in the low and high fluoride areas.

8.11 Daily urinary fluoride excretion (DUFE)

8.11.1 Children

Table 8.18 shows the mean corrected 24-h urine volumes and daily fluoride excretion by fluoride area for all the children for whom data were complete; those with incomplete 24-h urine were excluded. The mean urinary volume in the high fluoride area was higher than that of the low fluoride area and the difference between these means was statistically significant. The difference in means was also statistically significant between the fluoride areas when expressed on a bodyweight basis. There was a statistically significant difference in children's DUFE between low- and high fluoride areas.

Table 8.18 Mean (SD) Urinary fluoride concentration (UFC), corrected urine volume and DUFE by fluoride area for children

	Area		Mean difference (95% CI)	P value
	Low fluoride (n=28)	High fluoride (n=29)		
(UFC) (mg/l)	0.175 (0.111)	5.696 (3.075)	-5.521 (-6.691, -4.350)	<0.001
Corrected urine volume				
ml/d	445(197)	631 (418)	-186 (-359, -12)	0.036
ml/kg bw/d	27.79 (12.81)	42.21 (30.80)	-14.41 (-26.96, -1.87)	0.025
DUFE				
mg/d	0.071 (0.039)	3.110 (1.774)	-3.039 (-3.727, -2.351)	<0.001
mg/kgbw/d	0.004 (0.002)	0.210 (0.143)	-0.205 (-0.261, -0.150)	<0.001

DUFE (24-h urine samples)

8.11.2 Adults

The mean values of 24-h urinary volume and daily urinary fluoride excretion for adults by fluoride area are presented in Table 8.19. The corrected 24-h urinary volume and DUFE in the high fluoride area were statistically significantly higher than in the low fluoride area. Similarly, when corrected 24-h urine volume and DUFE were expressed on a body weight basis, the means in the high fluoride area were statistically significantly higher than in the low fluoride area.

Table 8.19 Mean (SD) UFC, corrected urine volume and DUFE by fluoride area for adults (n=55)

	Area		Mean difference (95% CI)	P value
	Low fluoride (n=28	High fluoride (n=27		
UFC (mg/l)	0.357 (0.228)	7.065 (4.470)	-6.708 (-8.443, -4.973)	<0.001
Corrected urine volume				
ml/d	1048 (474)	1441(567)	-393 (-669, -116)	0.006
ml/kg bw/d	15.80 (7.16)	22.12 (7.92)	-6.307 (-10.29, -2.32)	0.002
DUFE				
mg/d	0.355 (0.213)	8.658 (4.339)	-8.303 (-9.987, -6.619)	<0.001
mg/kgbw/d	0.005 (0.003)	0.140 (0.081)	-0.134 (-0.166, -0.103)	<0.001

8.12 Fluoride biomarkers

8.12.1 Children

Table 8.20 shows the mean (SD) and range of all the biomarkers among children living in the high- and low fluoride areas. There was a statistically significant difference ($p < 0.001$) between high- and low fluoride areas for all the biological markers. In the low fluoride area, the concentration of fluoride in toenails was slightly higher than in fingernails on average, whereas, in the high fluoride area, fingernail fluoride was slightly higher than toenail fluoride. There was a wide variation in the fluoride concentrations of the biomarkers in both low- and high fluoride areas.

8.12.2 Adults

Table 8.21 shows the mean (SD) and range of all the biomarkers among adults living in the high- and low fluoride areas. There was a statistically significant difference between high- and low fluoride areas for all the biological markers. Here, the fluoride concentration of toenails was higher than that of the fingernails in both low- and high fluoride areas compared

to results obtained with the children. There was a wide variation in the fluoride concentrations of the biomarkers in both low- and high fluoride areas.

Table 8.20 Comparison of the mean (SD) of Daily urinary fluoride excretion, fluoride concentration of saliva, plasma, hair and nails (fingernail and toenail) by children in low- and high fluoride water areas

	Area		Mean difference (95% CI)	P value
	Low fluoride	High fluoride		
Daily Urinary fluoride excretion (DUFE)				
mg/d	0.071 (0.039)	3.110 (1.774)	-3.039 (-3.727, -2.351)	<0.001
mg/kgbw/d	0.004 (0.002)	0.210 (0.143)	-0.205 (-0.261, -0.150)	<0.001
Saliva fluoride				
µmol/l	0.405 (0.255)	16.012 (19.383)	-15.607 (-22.845, -8.368)	<0.001
ng/ml	8.393 (5.990)	304.23 (368.28)	-295.84 (-433.37, -158.30)	<0.001
Plasma fluoride				
µmol/l	1.504 (0.565)	6.081 (2.658)	-4.577 (-5.588, -3.566)	<0.001
ng/ml	28.57 (10.73)	115.53 (50.51)	-86.97 (-106.18, -67.75)	<0.001
Hair (µg/g)	0.743 (0.609)	1.831 (1.091)	-1.088 (-1.590, -0.586)	<0.001
Fingernail (µg/g)	3.237 (2.636)	12.583 (7.057)	-9.437 (-12.505, -6.188)	<0.001
Toenail (µg/g)	3.957 (3.704)	11.381 (5.232)	-7.423 (-9.905, -4.941)	<0.001

Table 8.21 Comparison of the mean (SD) of 24-h urinary fluoride excretion, fluoride concentration of saliva, plasma, hair and nails (fingernail and toenail) by adults in low- and high fluoride water areas

	Area		Mean difference (95% CI)	P value
	Low fluoride	High fluoride		
Daily urinary fluoride excretion (DUFE)				
mg/d	0.355 (0.213)	8.658 (4.339)	-8.303 (-9.967, -6.619)	<0.001
mg/kgbw/d	0.005 (0.003)	0.140 (0.081)	-0.134 (-0.166, -0.103)	<0.001
Saliva fluoride				
µmol/l	0.452 (0.485)	12.595 (16.916)	-12.143 (-18.462, -5.825)	<0.001
ng/ml	8.581 (9.215)	239.3 (321.4)	-230.7 (-350.7, -110.7)	<0.001
Plasma fluoride				
µmol/l	1.618 (0.747)	6.674 (2.737)	-5.055 (-6.107, -4.004)	<0.001
ng/ml	30.75 (14.19)	126.80 (52.00)	-96.05 (-116.02, -76.07)	<0.001
Hair (µg/g)	1.368 (1.062)	5.690 (3.182)	-4.323 (-5.634, -3.011)	<0.001
Fingernail (µg/g)	2.805 (1.776)	9.415 (3.749)	-6.609 (-8.227, -4.992)	<0.001
Toenail (µg/g)	3.286 (2.823)	10.207 (5.792)	-6.921 (-9.440, -4.401)	<0.001

8.13 Comparison between children and adults

8.13.1 Low fluoride area

The comparison of total daily fluoride intake and fluoride concentration of biomarkers in the low fluoride area is presented in Table 8.22. Total daily fluoride intake was higher for adults than for children. However, when these values were adjusted by weight, the total daily fluoride intake of children was higher compared to that of adults. Consequently, daily intake from diet as well as toothpaste in mg/kgbw/d was higher for children than for adults. There was a statistically significant difference between fluoride intake by children and by adults.

Fluoride concentrations in saliva and plasma were similar for children and adults. Daily urinary fluoride excretion when adjusted by bodyweight was also similar for adults and children. The concentration of fluoride in adults' hair was twice the concentration in children. There were no statistically significant differences for all the biomarkers between children and adults except for hair which was statistically significant ($p = 0.009$).

8.13.2 High fluoride area

The comparison of total daily fluoride intake and F concentration of biomarkers in the high fluoride area is presented in Table 8.23. There was a statistically significant difference in the total daily fluoride intake between adults and children. However, there were no statistically significant differences for the biomarkers between children and adults except for where the hair fluoride concentration in adults was three times that of the children on average and for urinary fluoride excretion (mg/d and mg/kgbw/d).

Table 8.22 Comparison of the mean (SD) of fluoride intake, fluoride excretion and fluoride biomarkers in children and adults living in the low fluoride area

Fluoride intake from:	age group		Mean difference (95% CI)	P value
	Children	Adults		
Diet				
mg/d	0.984 (0.603)	1.904 (1.218)	-0.919 (-1.403, -0.435)	<0.001
mg/kgbw/d	0.060 (0.038)	0.029 (0.019)	+0.031 (+0.016, +0.046)	<0.001
Toothpaste ingestion				
mg/d	0.229 (0.135)	0.416 (0.335)	-0.188 (-0.317, -0.059)	0.005
mg/kgbw/day	0.014 (0.008)	0.006 (0.005)	-0.008 (-0.011, -0.005)	<0.001
TDFI (toothpaste and diet)				
mg/d	1.214 (0.575)	2.321 (1.278)	-1.107 (-1.607, -0.607)	<0.001
mg/kgbw/d	0.075 (0.036)	0.036 (0.020)	0.038 (+0.024, +0.053)	<0.001
Fluoride Excretion				
Corrected urine volume				
ml/d	445 (197)	1048 (474)	-604 (-794, -414)	<0.001
ml/kgbw/d	27.79 (12.81)	15.80 (7.16)	+11.99 (+6.520, +17.466)	<0.001
DUFE				
mg/d	0.071 (0.039)	0.355 (0.213)	-0.284 (-0.368, -0.200)	<0.001
mg/kgbw/d	0.004 (0.002)	0.005 (0.003)	-0.001 (-0.003, +0.001)	0.194
Fluoride concentration of:				
Saliva (µg/ml)	0.008 (0.006)	0.009 (0.009)	-0.000 (-0.004, +0.004)	0.926
Plasma (µg/ml)	0.029 (0.011)	0.031 (0.014)	-0.002 (-0.009, +0.004)	0.496
Fingernail (µg/g)	3.237 (2.636)	2.805 (1.776)	0.431 (-0.903, +1.766)	0.516
Toenail (µg/g)	4.073 (3.728)	3.286 (2.823)	0.787 (-1.059, +2.632)	0.395
Hair (µg/g)	0.743 (0.609)	1.368(1.062)	-0.625 (-1.087, -0.163)	0.009

Table 8.23 Comparison of the mean (SD) of fluoride intake, fluoride excretion and fluoride biomarkers in children and adults living in the high fluoride area

Fluoride intake from:	age group		Mean difference (95% CI)	P value
	Children	Adults		
Diet				
mg/d	3.738 (2.184)	7.515 (5.810)	-3.776 (-6.115, -1.438)	0.002
mg/kgbw/d	0.257 (0.186)	0.116 (0.096)	+0.146 (+0.069, +2.223)	<0.001
Toothpaste ingestion				
mg/d	0.153 (0.170)	0.362 (0.332)	-0.209 (-0.346, -0.072)	0.004
mg/kgbw/day	0.014 (0.016)	0.007 (0.004)	-0.007 (-0.014, +0.000)	0.055
TDFI (toothpaste and diet)				
mg/d	3.896 (2.147)	7.889 (5.721)	-3.993 (-6.295, -1.691)	0.001
mg/kgbw/d	0.277 (0.184)	0.125 (0.093)	+0.151 (+0.074, +0.229)	<0.001
Fluoride Excretion				
Corrected urine volume				
ml/d	631 (418)	1440 (567)	-810 (-1076, -545)	<0.001
ml/kgbw/d	42.21 (30.80)	22.11 (7.92)	+20.10 (+8.055, +32.145)	0.002
DUFE				
mg/d	3.110 (1.774)	8.658 (4.339)	-5.548 (-7.345, -3.751)	<0.001
mg/kgbw/d	0.210 (0.143)	0.140 (0.081)	+0.070 (+0.007, +0.133)	0.029
Fluoride concentration of:				
Saliva (µg/ml)	0.304 (0.368)	0.239 (0.321)	+0.065 (-0.114, +0.244)	0.470
Plasma (µg/ml)	0.116 (0.051)	0.127 (0.052)	-0.011 (-0.038, +0.015)	0.398
Fingernail (µg/g)	12.583 (7.057)	9.415 (3.749)	+3.169 (-0.105, +6.442)	0.057
Toenail (µg/g)	11.381 (5.232)	10.207 (5.792)	+1.174 (-1.841, +4.189)	0.438
Hair (µg/g)	1.831 (1.091)	5.691(3.182)	-3.859 (-5.183, -2.536)	<0.001

8.14 Correlations between fluoride exposure and fluoride biomarkers

The correlation between total daily fluoride intake (diet and toothpaste) (mg/kgbw/d) and

- Daily urinary fluoride excretion (mg/kgbw/d) in children was statistically significantly positive and strong (Pearson's correlation = 0.756, $p < 0.001$). Similarly, a statistically significant positive correlation (Pearson's correlation = 0.629, $p < 0.001$) was found in adults (Figure 8.3).
- Saliva fluoride concentration (µg/ml) was weak but statistically significantly positive (Pearson's correlation = 0.376, $p = 0.004$) in children. Conversely, no statistically significant correlation (Pearson's correlation = 0.195, $p = 0.136$) was found in adults (Figure 8.5).
- Plasma fluoride concentration (µg/ml) in children was statistically significantly positive and strong (Pearson's correlation = 0.770, $p < 0.001$). Similarly, a statistically significant positive correlation (Pearson's correlation = 0.745, $p < 0.001$) was found in adults (Figure 8.7).
- Fingernail fluoride concentration was moderate and strong, positive and statistically significant in children (Pearson's correlation = 0.448, $p = 0.002$) and in adults (Pearson's correlation = 0.506, $p < 0.001$) (Figure 8.9).
- Toenail fluoride concentration was moderate and strong, positive and statistically significant in children (Pearson's correlation = 0.488, $p < 0.001$) and in adults (Pearson's correlation = 0.502, $p < 0.001$) (Figure 8.11).
- Hair fluoride concentration was weak but statistically significantly positive (Pearson's correlation = 0.306, $p = 0.027$) in children. However, the correlation was strong, positive and statistically significant in adults (Pearson's correlation = 0.605, $p < 0.001$) (Figure 8.13).

The correlation between fluoride in supply water (mg/l) and

- Daily urinary fluoride excretion (mg/kgbw/d) in children was statistically significantly positive and strong (Pearson's correlation = 0.785, $p < 0.001$). Similarly, a statistically significantly positive correlation (Pearson's correlation = 0.853, $p < 0.001$) was found in adults (Figure 8.4).
- Saliva fluoride concentration (µg/ml) was moderate and statistically significantly positive (Pearson's correlation = 0.547, $p < 0.001$) in children. Conversely, a weak,

statistically significant correlation (Pearson's correlation = 0.372, p = 0.003) was found in adults (Figure 8.6).

- Plasma fluoride concentration (μg/ml) in children was statistically significantly positive and very strong (Pearson's correlation = 0.825, p < 0.001). Similarly, a statistically significantly positive correlation (Pearson's correlation = 0.858, p < 0.001) was found in adults (Figure 8.8).
- Fingernail fluoride concentration was moderate, positive and statistically significant in children (Pearson's correlation = 0.599, p < 0.001) and in adults, a strong, positive statistically significant correlation (Pearson's correlation = 0.732, p < 0.001) was found (Figure 8.10).
- Toenail fluoride concentration was strong, positive and statistically significant in children (Pearson's correlation = 0.657, p < 0.001) and in adults (Pearson's correlation = 0.660, p < 0.001) (Figure 8.12).
- Hair fluoride concentration was moderate but statistically significantly positive (Pearson's correlation = 0.420, p = 0.002) in children. However, the correlation was strong, positive and statistically significant in adults (Pearson's correlation = 0.689, p < 0.001) (Figure 8.14).

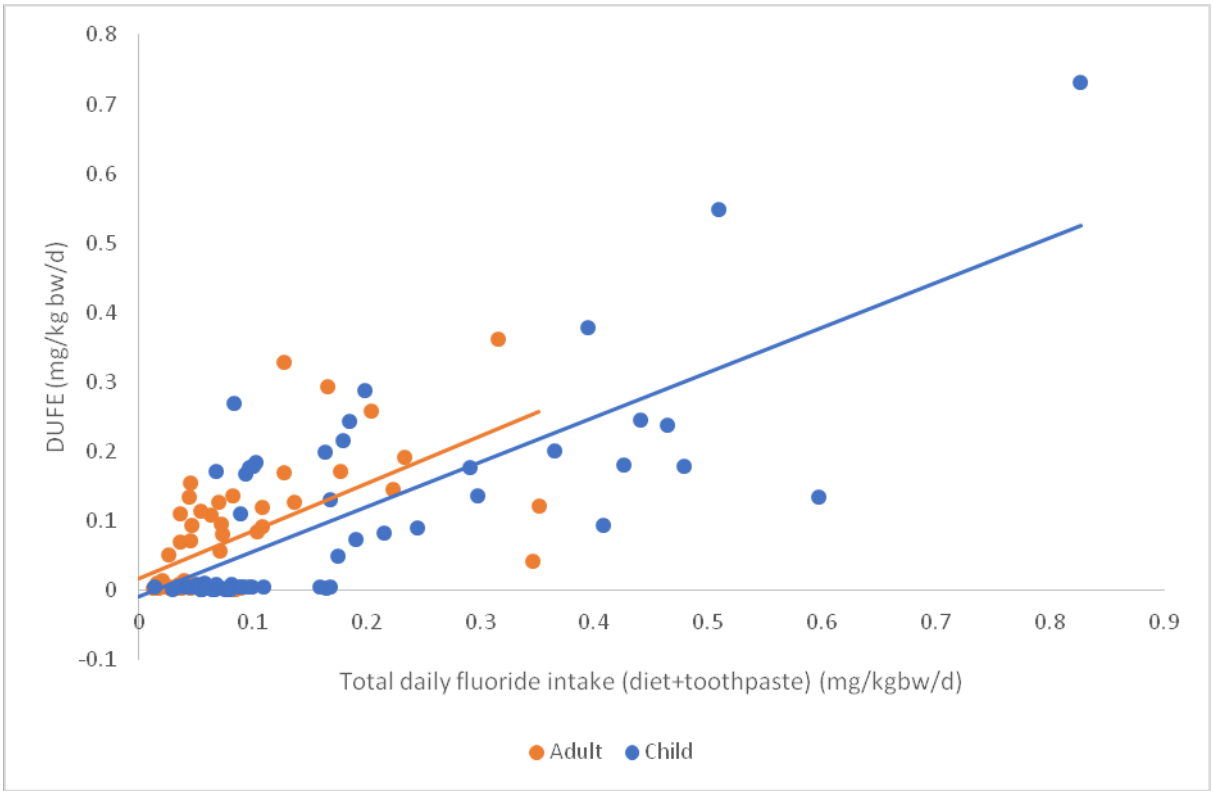

Figure 8.3 Relationship between daily urinary fluoride excretion (DUFE) and total daily fluoride intake (TDFI). *[DUFE (mg/kgbw/d) =-0.008 + [0.647 x TDFI (mg/kgbw/d)] (children)**;** DUFE (mg/kgbw/d) =0.017 + [0.687 x TDFI (mg/kgbw/d)] (adults)]*

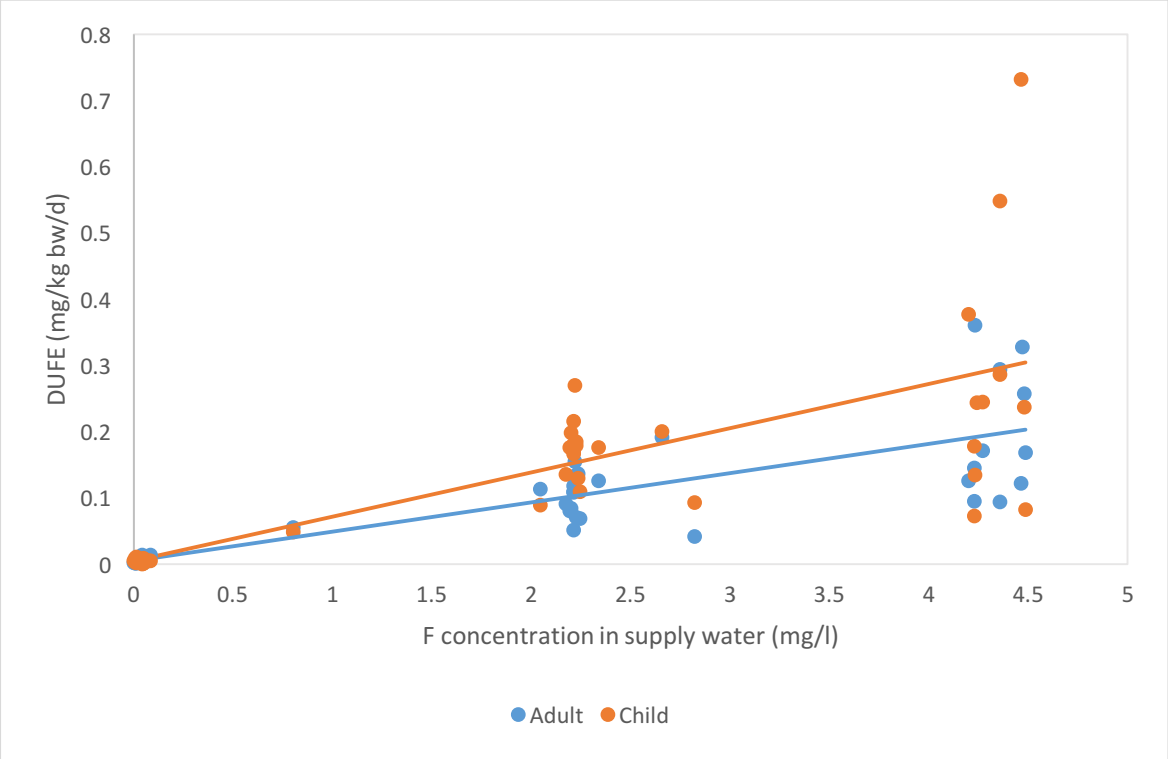

Figure 8.4 Relationship between daily urinary fluoride excretion (DUFE) and fluoride concentration in drinking water. *[DUFE (mg/kgbw/d) =0.005 + [0.067 x Drinking water fluoride (mg/l)] (children); DUFE (mg/kgbw/d) =0.005 + [0.044 x Drinking water fluoride (mg/l)] (adults)]*

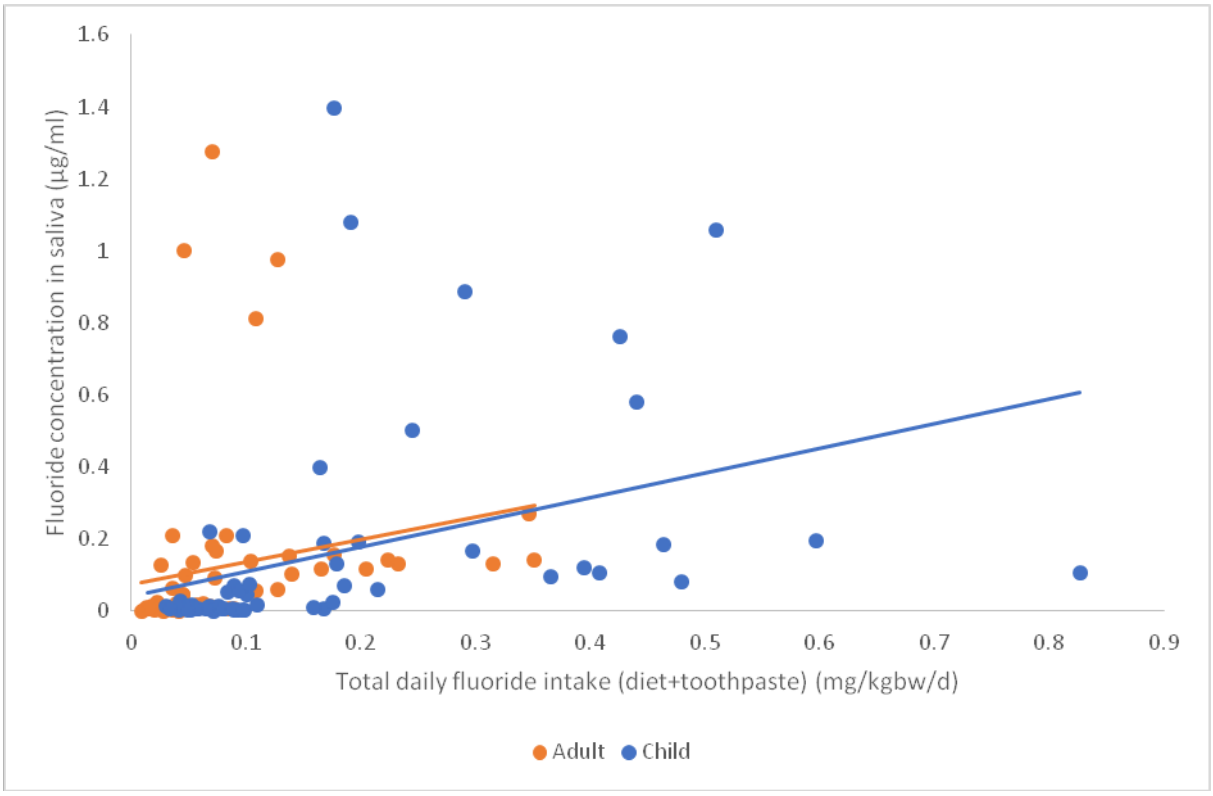

Figure 8.5 Relationship between saliva fluoride concentration and total daily fluoride intake (TDFI). **[***Saliva fluoride concentration (µg/ml) =0.043 + [0.684 x TDFI (mg/kgbw/d)] (child); Saliva fluoride concentration (µg/ml) =0.075 + [0.620 x TDFI (mg/kgbw/d)] (adult)]*

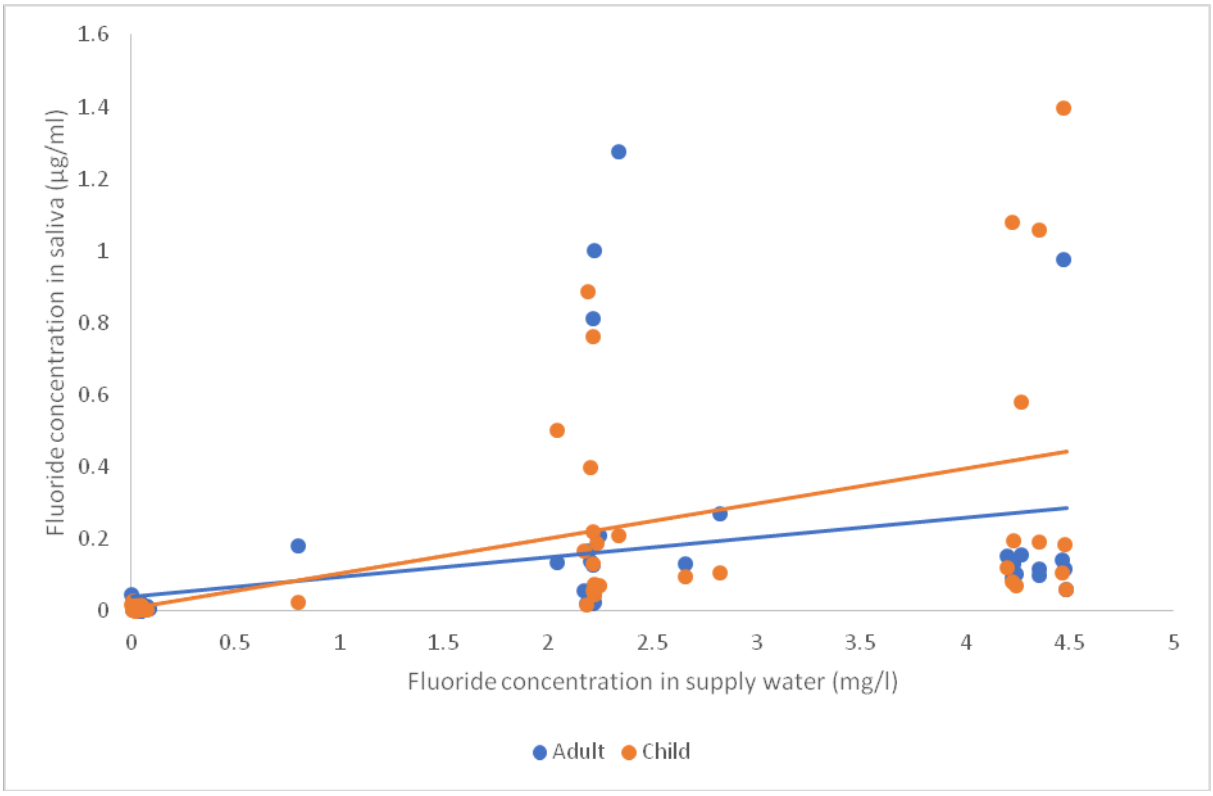

Figure 8.6 Relationship between saliva fluoride concentration and fluoride concentration in drinking water. **[***Saliva fluoride concentration (µg/ml) =0.007 + [0.097 x Drinking water fluoride (mg/l)] (children); Saliva fluoride concentration (µg/ml) =0.039 + [0.055 x Drinking water fluoride (mg/l)] (adults)]*

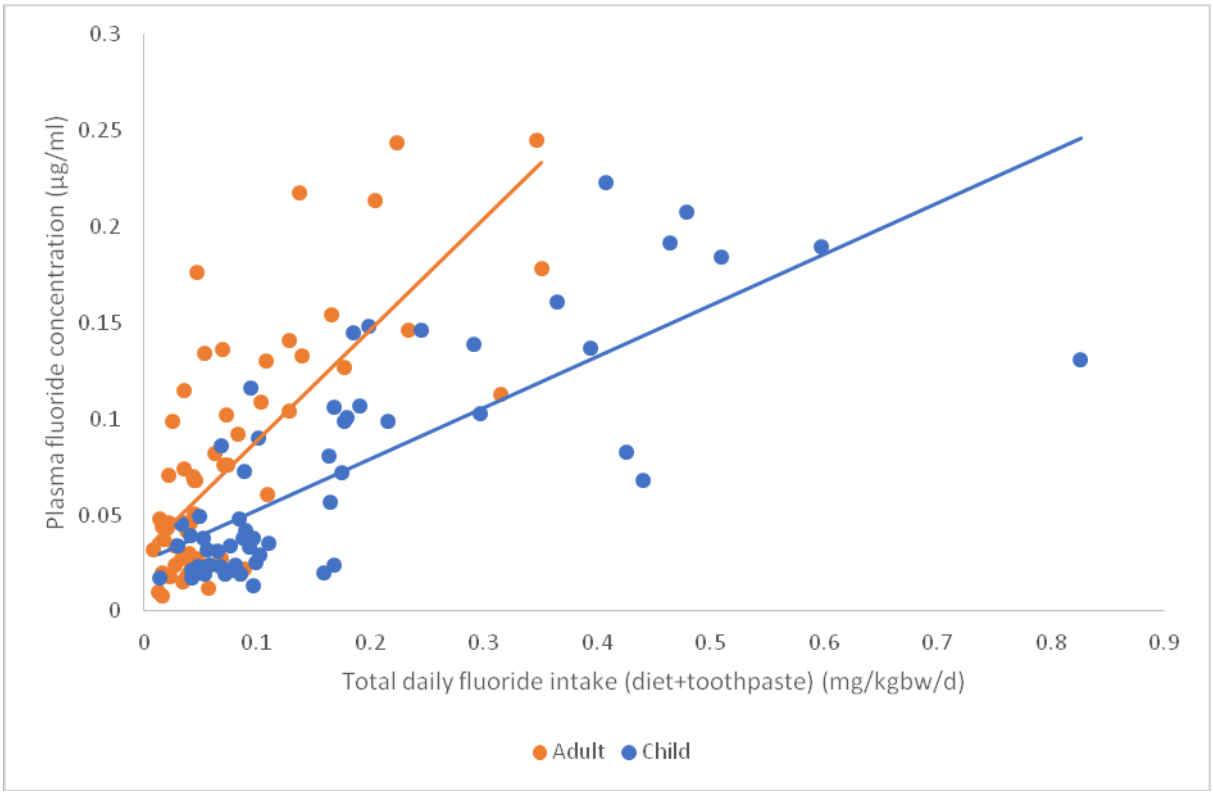

Figure 8.7 Relationship between plasma fluoride concentration and total daily fluoride intake (TDFI). **[***Plasma fluoride concentration (µg/ml) =0.026 + [0.267 x TDFI (mg/kgbw/d)] (child); Plasma fluoride concentration (µg/ml) =0.031 + [0.576 x TDFI (mg/kgbw/d) (adult)]*

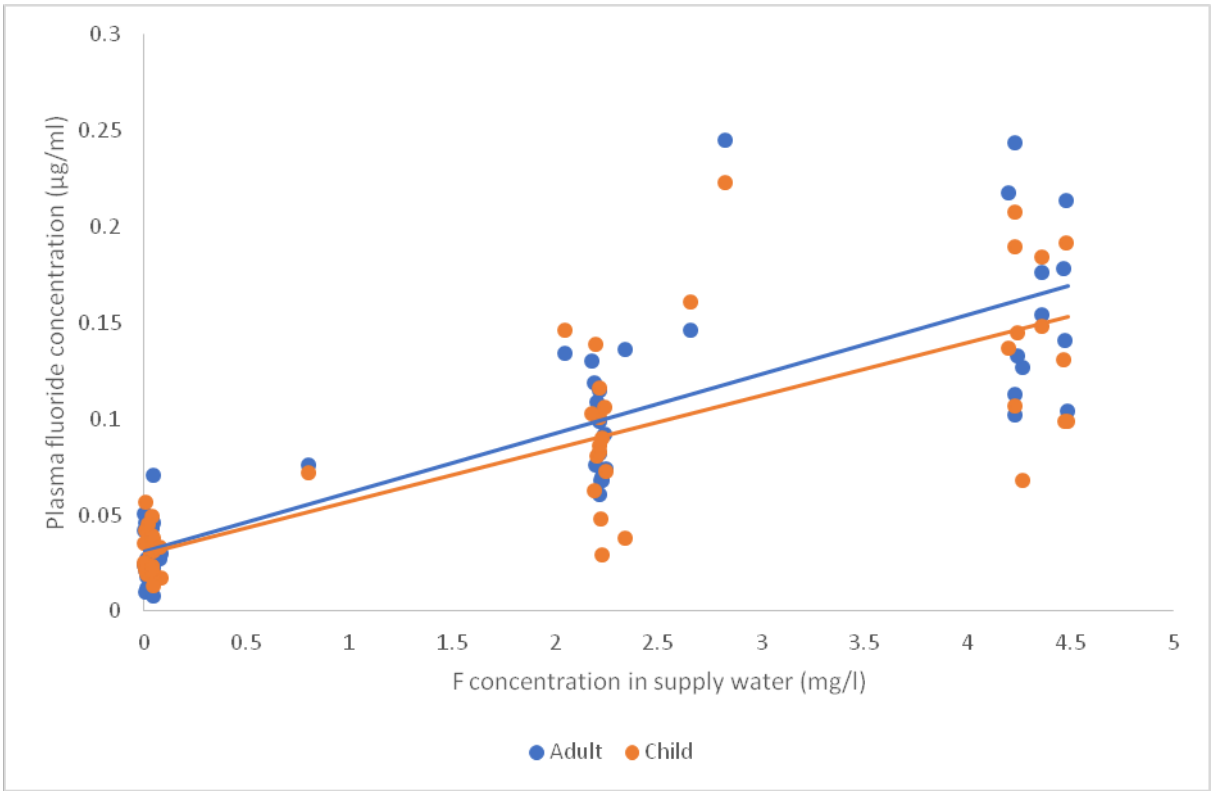

Figure 8.8 Relationship between plasma fluoride concentration and fluoride concentration in drinking water**. [***Plasma fluoride concentration (µg/ml) =0.030 + [0.028 x Drinking water fluoride (mg/l) (children); Plasma fluoride concentration (µg/ml) =0.031 + [0.0.031 x Drinking water fluoride (mg/l) (adults)]*

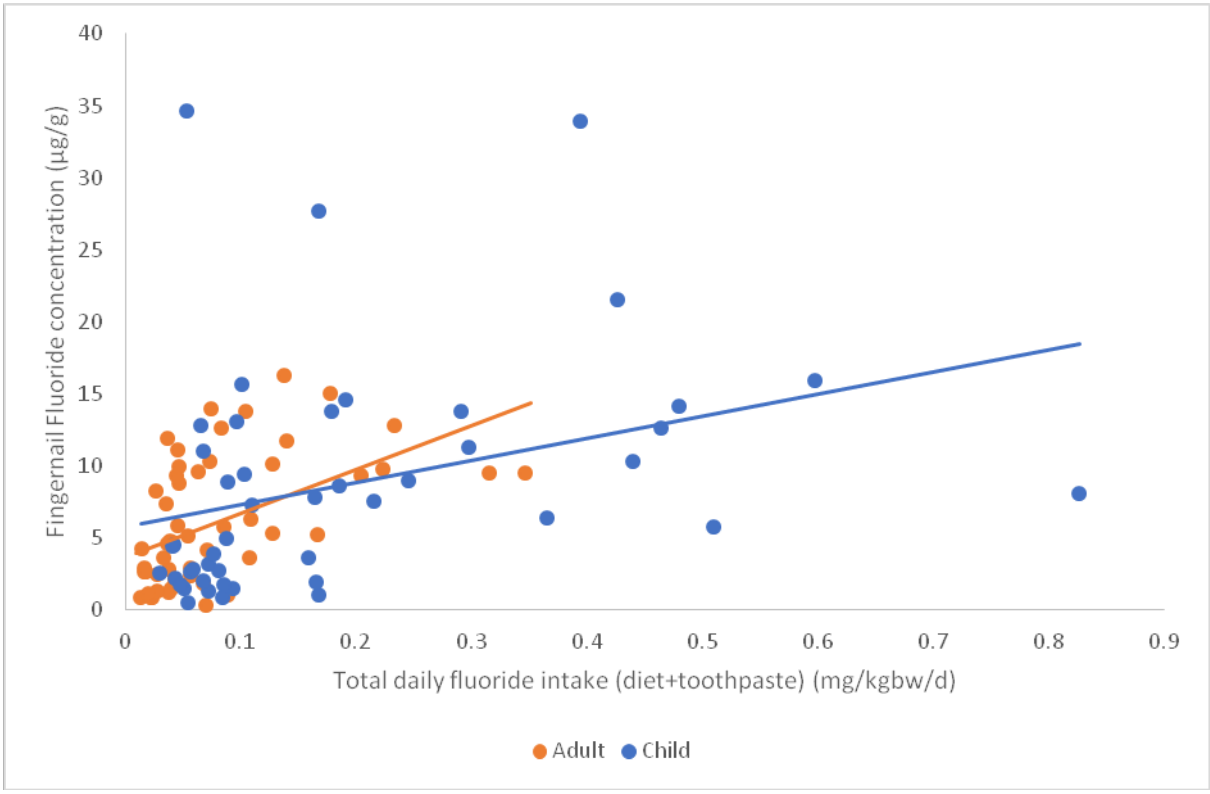

Figure 8.9 Relationship between fingernail fluoride concentration and total daily fluoride intake (TDFI). **[***Fingernail fluoride concentration (µg/g) =5.82 + [15.33 x TDFI (mg/kgbw/d)] (children); Fingernail fluoride concentration (µg/g) =3.71 + [30.45 x TDFI (mg/kgbw/d)] (adults)]*

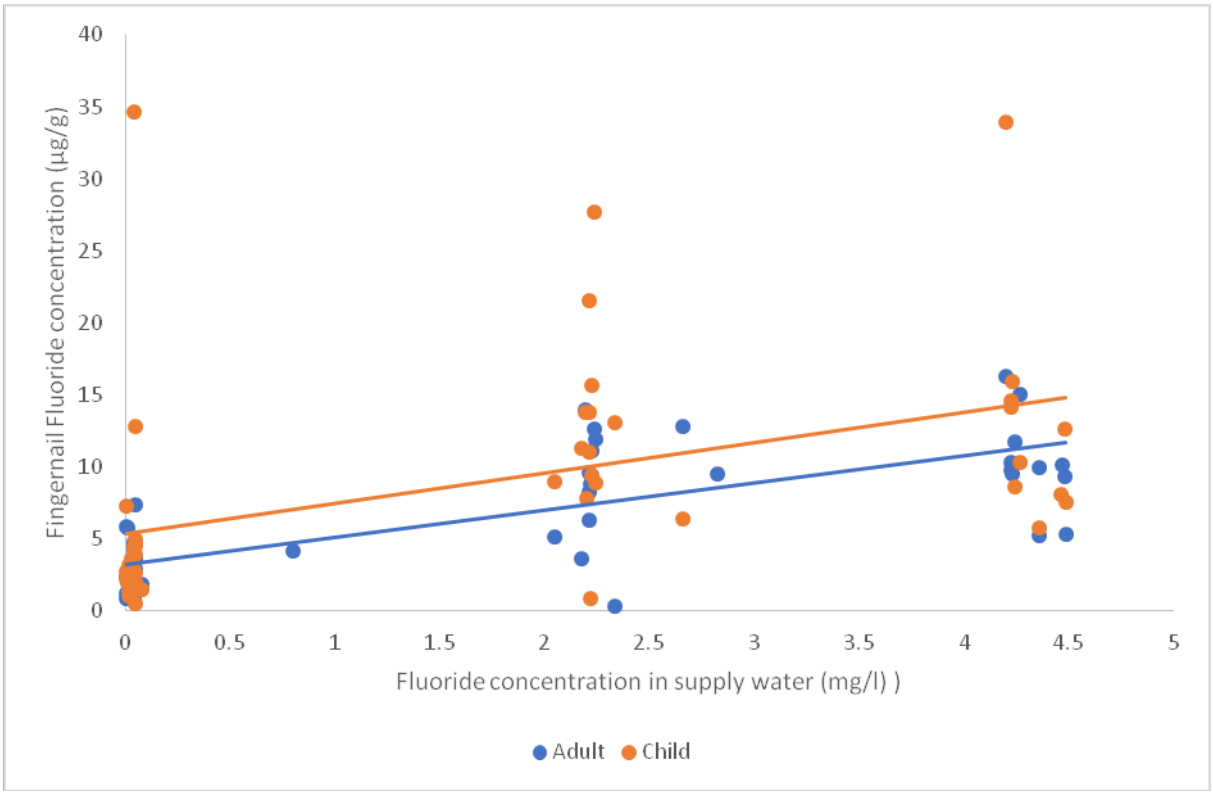

Figure 8.10 Relationship between fingernail fluoride concentration and fluoride concentration in drinking water. *[Fingernail fluoride concentration (µg/g) =5.33 + [2.13 x Drinking water fluoride (mg/l)] (children); Fingernail fluoride concentration (µg/g) =3.20 + [1.89 x Drinking water fluoride (mg/l)] (adults)]*

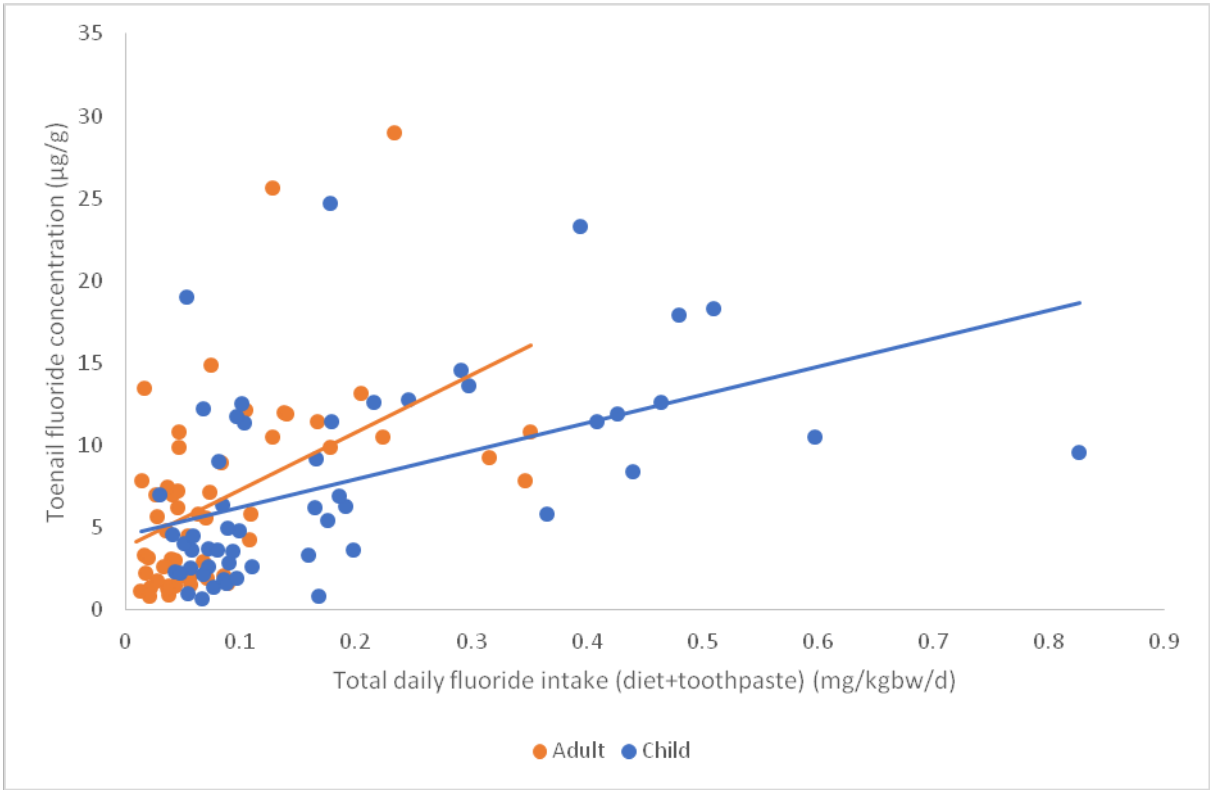

Figure 8.11 Relationship between toenail fluoride concentration and total daily fluoride intake (TDFI). *Toenail fluoride concentration (µg/g) =4.51 + [17.17 x TDFI (mg/kgbw/d)] (children); Toenail fluoride concentration (µg/g) =3.82 + [34.91 x TDFI (mg/kgbw/d)] (adults)*.

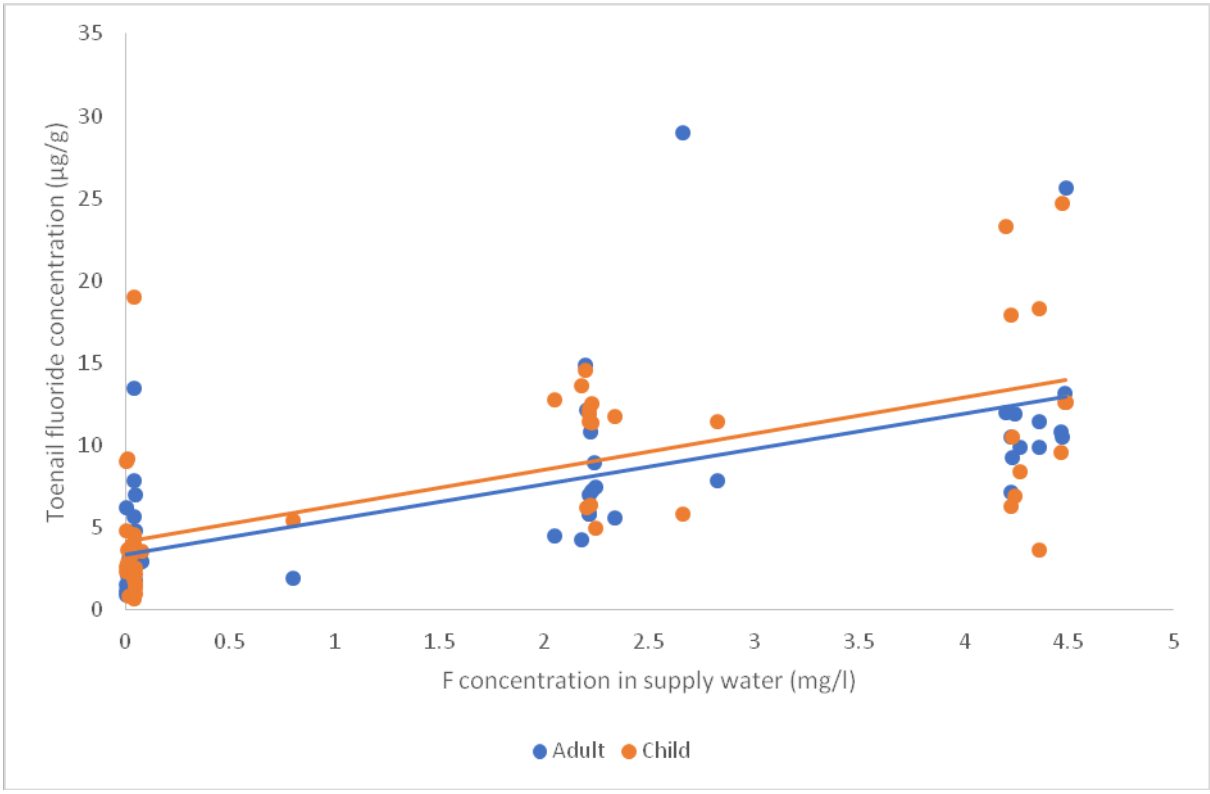

Figure 8.12 Relationship between toenail fluoride concentration and fluoride concentration in drinking water. *[Toenail fluoride concentration (µg/g) =4.13 + [2.20 x Drinking water fluoride (mg/l)] (children); Toenail fluoride concentration (µg/g) =3.82 + [3.36 x Drinking water fluoride (mg/l)] (adults)*]

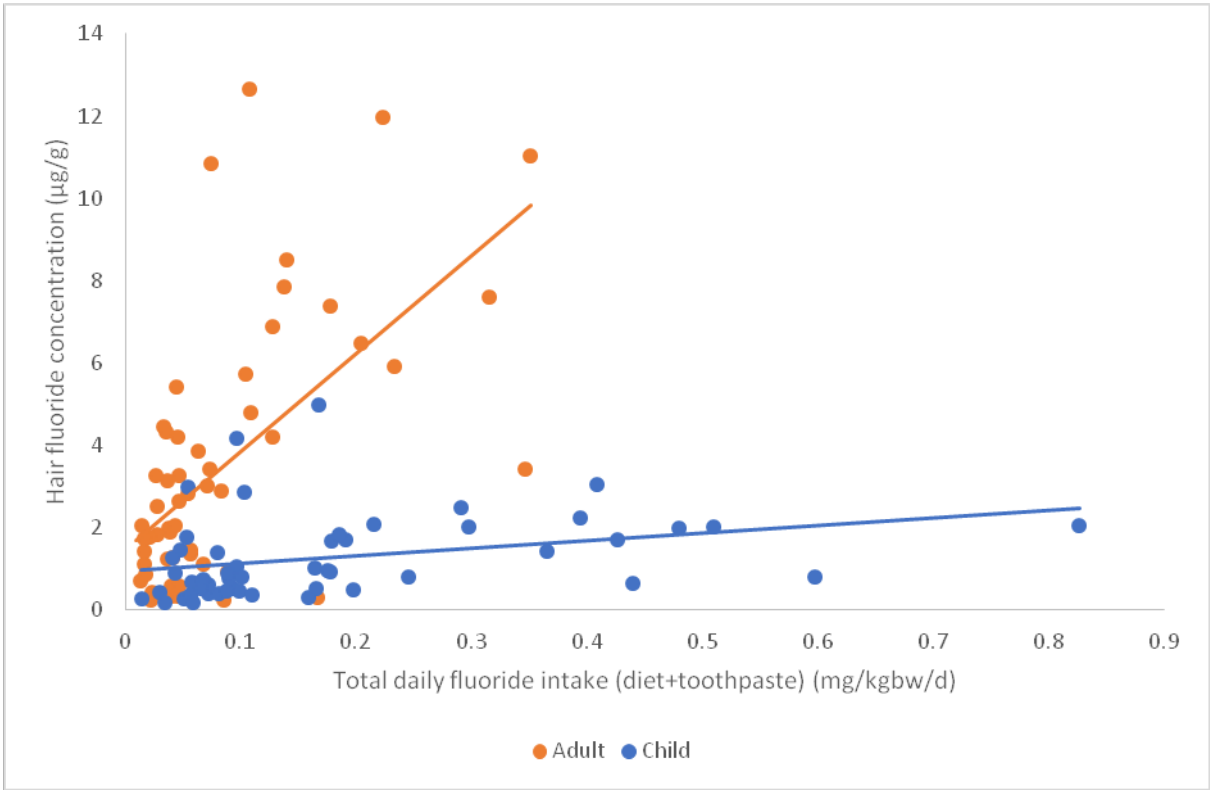

Figure 8.13 Relationship between hair fluoride concentration and total daily fluoride intake (TDFI). **[***Hair fluoride concentration (µg/g) =0.94 + [1.86 x TDFI (mg/kgbw/d)] (children); Hair fluoride concentration (µg/g) =1.49 + [23.73 x TDFI (mg/kgbw/d)] (adults)]*

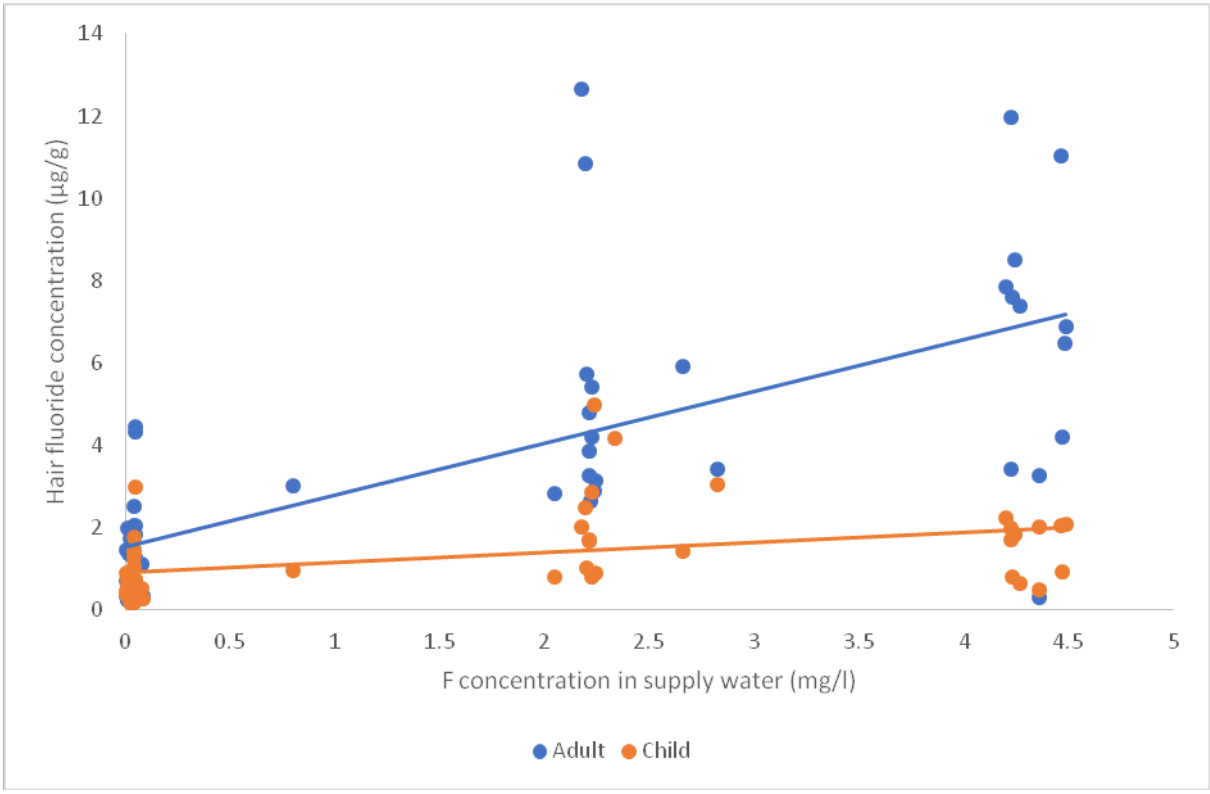

Figure 8.14 Relationship between hair fluoride concentration and fluoride concentration in drinking water. *[Hair fluoride concentration (µg/g) =0.90 + [0.25 x Drinking water fluoride (mg/l)] (children); Hair fluoride concentration (µg/g) =1.55 + [1.25 x Drinking water fluoride (mg/l)] (adults)*]

Summary

Table 8.24 presents the Pearson correlation coefficient and associated p value for the relationships between each biomarker and total daily fluoride intake for adults and children. There were positive correlations for all the biomarkers in children. In adults and children, there were weak correlations (r = 0.195 and r=376 respectively) between saliva and TDFI but there were moderate to strong correlations for the other biomarkers: plasma, toenail, fingernail, hair and urine, except in children where a weak correlation was found for hair and TDFI. Table 8.25 presents the Pearson correlation coefficient and associated p value for the relationships between each biomarker and fluoride concentration in supply drinking water for adults and children. There were moderate to strong correlations for plasma, toenails, fingernails, hair and urine biomarkers for both adults and children. In saliva, a weak correlation was found for adults whereas a moderate correlation was found for children.

Table 8.24 Summary of relationships between fluoride biomarkers and total daily fluoride intake (mg/kgbw/d)

Fluoride Biomarker	Pearson correlation (p value)	
	Children	Adults
Saliva	0.376 (p=0.004)	0.195 (p=0.136)
Plasma	0.770 (p<0.001)	0.745 (p<0.001)
Toenail	0.488 (p<0.001)	0.502 (p<0.001)
Fingernail	0.448 (p=0.002)	0.506 (p<0.001)
Hair	0.306 (p=0.027)	0.605 (p<0.001)
Urine	0.756 (p<0.001)	0.629 (p<0.001)

Table 8.25 Summary of relationships between fluoride biomarkers and fluoride concentration in drinking water

Fluoride Biomarker	Pearson correlation (p value)	
	Children	Adults
Saliva	0.547 (p<0.001)	0.372 (p=0.003)
Plasma	0.825 (p<0.001)	0.858 (p<0.001)
Toenail	0.657 (p<0.001)	0.660 (p<0.001)
Fingernail	0.599 (p<0.001)	0.732 (p<0.001)
Hair	0.420 (p=0.002)	0.689 (p<0.001)
Urine	0.785 (p<0.001)	0.853 (p<0.001)

8.15 Correlations between biomarkers

The correlation between daily urinary fluoride excretion (mg/kgbw/d) and

- Saliva fluoride concentration (µg/ml) was moderate, statistically significantly positive in children (Pearson's correlation = 0.424, p = 0.002) and in adults (Pearson's correlation = 0.460, p < 0.001) (Figure 8.15).
- Plasma fluoride concentration (µg/ml) in children was statistically significantly positive and strong (Pearson's correlation = 0.621, p < 0.001). Similarly, a strong statistically significant positive correlation (Pearson's correlation = 0.671, p < 0.001) was found in adults (Figure 8.16).
- Fingernail fluoride concentration was moderate, positive and statistically significant in children (Pearson's correlation = 0.401, p = 0.008) and a moderate, positive statistically significant correlation (Pearson's correlation = 0.551, p < 0.001) was found in adults (Figure 8.17).
- Toenail fluoride concentration was moderate, positive and statistically significant in children (Pearson's correlation = 0.542, p < 0.001) and in adults (Pearson's correlation = 0.559, p < 0.001) (Figure 8.18).
- Hair fluoride concentration was weak but statistically significantly positive (Pearson's correlation = 0.383, p = 0.006) in children. However, the correlation was moderate, positive and statistically significant in adults (Pearson's correlation = 0.520, p < 0.001) (Figure 8.19).

The correlation between fluoride concentration in saliva (µg/ml) and

- Plasma fluoride concentration (µg/ml) was moderate, yet statistically significantly positive in children (Pearson's correlation = 0.405, p = 0.002) and a weak statistically significant correlation in adults (Pearson's correlation = 0.316, p = 0.013) (Figure 8.20).
- Fingernail fluoride concentration (µg/g) was weak, yet statistically significantly positive in children (Pearson's correlation = 0.349, p = 0.022) but not statistically significant in adults (Pearson's correlation = 0.172, p = 0.219) (Figure 8.21).
- Toenail fluoride concentration (µg/g) was moderate and statistically significantly positive in children (Pearson's correlation = 0.534, p < 0.001) but not statistically significant in adults (Pearson's correlation = 0.205, p = 0.144) (Figure 8.22).

- Hair fluoride concentration (µg/g) was not statistically significant in children (Pearson's correlation = 0.178, p = 0.221) and in adults (Pearson's correlation = 0.197, p = 0.149) (Figure 8.23).

The correlation between fluoride concentration in plasma (µg/ml) and

- Hair fluoride concentration was weak, yet statistically significantly positive (Pearson's correlation = 0.373, p = 0.008) in children. However, the correlation was strong, positive and statistically significant in adults (Pearson's correlation = 0.657, p < 0.001) (Figure 8.24).
- Toenail fluoride concentration was moderate, positive and statistically significant in children (Pearson's correlation = 0.593, p < 0.001) and in adults (Pearson's correlation = 0.568, p < 0.001) (Figure 8.25).
- Fingernail fluoride concentration was moderate, positive and statistically significant in children (Pearson's correlation = 0.552, p < 0.001) and a strong, positive statistically significant correlation (Pearson's correlation = 0.617, p < 0.001) was found in adults (Figure 8.26).

The correlation between fingernail fluoride concentration (µg/g) and

- Toenail fluoride concentration (µg/g) in children was statistically significantly positive and strong (Pearson's correlation = 0.760, p < 0.001). Similarly, a strong statistically significantly positive correlation (Pearson's correlation = 0.611, p < 0.001) was found in adults (Figure 8.27).
- Hair fluoride concentration was moderate, positive and statistically significant in children (Pearson's correlation = 0.542, p < 0.001) and a strong, positive statistically significant correlation (Pearson's correlation = 0.627, p < 0.001) was found in adults (Figure 8.28).

The correlation between toenail fluoride concentration (µg/g) and hair fluoride concentration was moderate and statistically significantly positive in children (Pearson's correlation = 0.534, p < 0.001) and in adults (Pearson's correlation = 0.528, p < 0.001) (Figure 8.29).

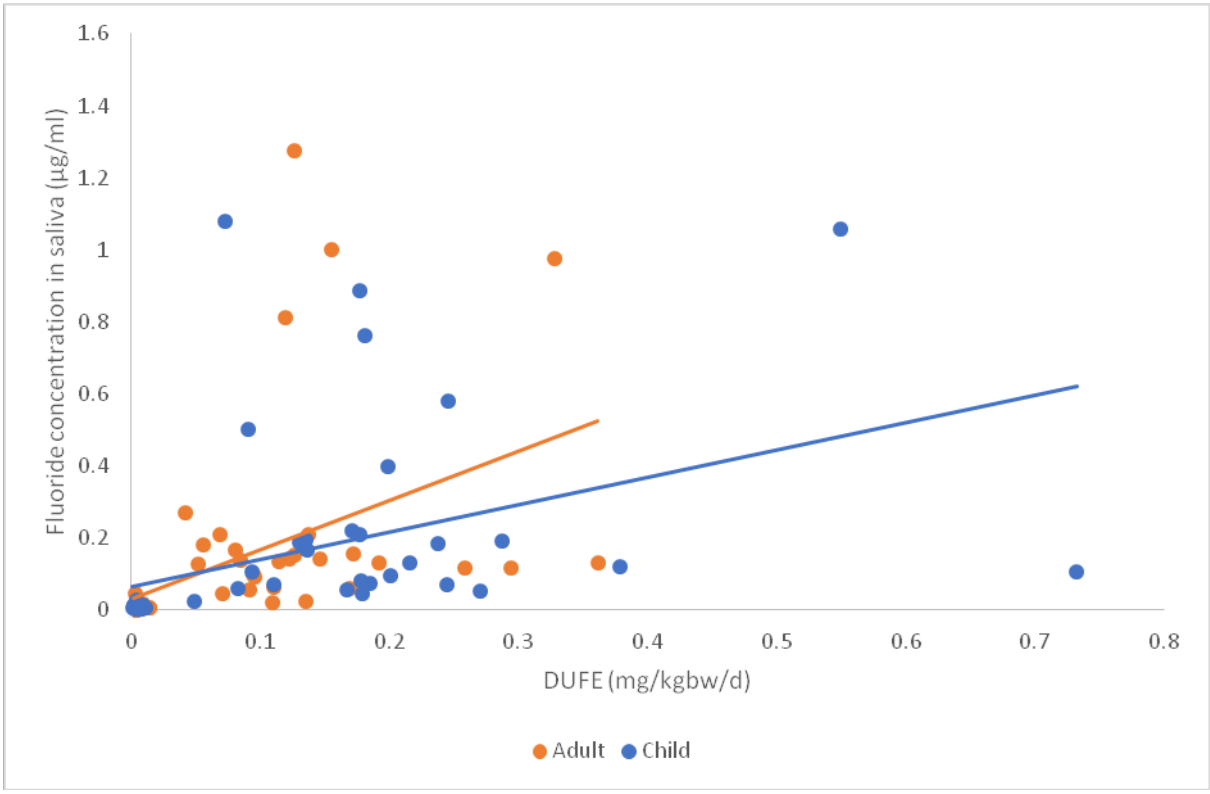

Figure 8.15 Relationship between saliva fluoride concentration and daily urinary fluoride excretion (DUFE). **[***Saliva fluoride concentration (µg/ml) =0.064 + [0.76 x DUFE (mg/kgbw/d)] (children); Saliva fluoride concentration (µg/ml) =0.032 + [1.36 x DUFE (mg/kgbw/d)] (adults)]*

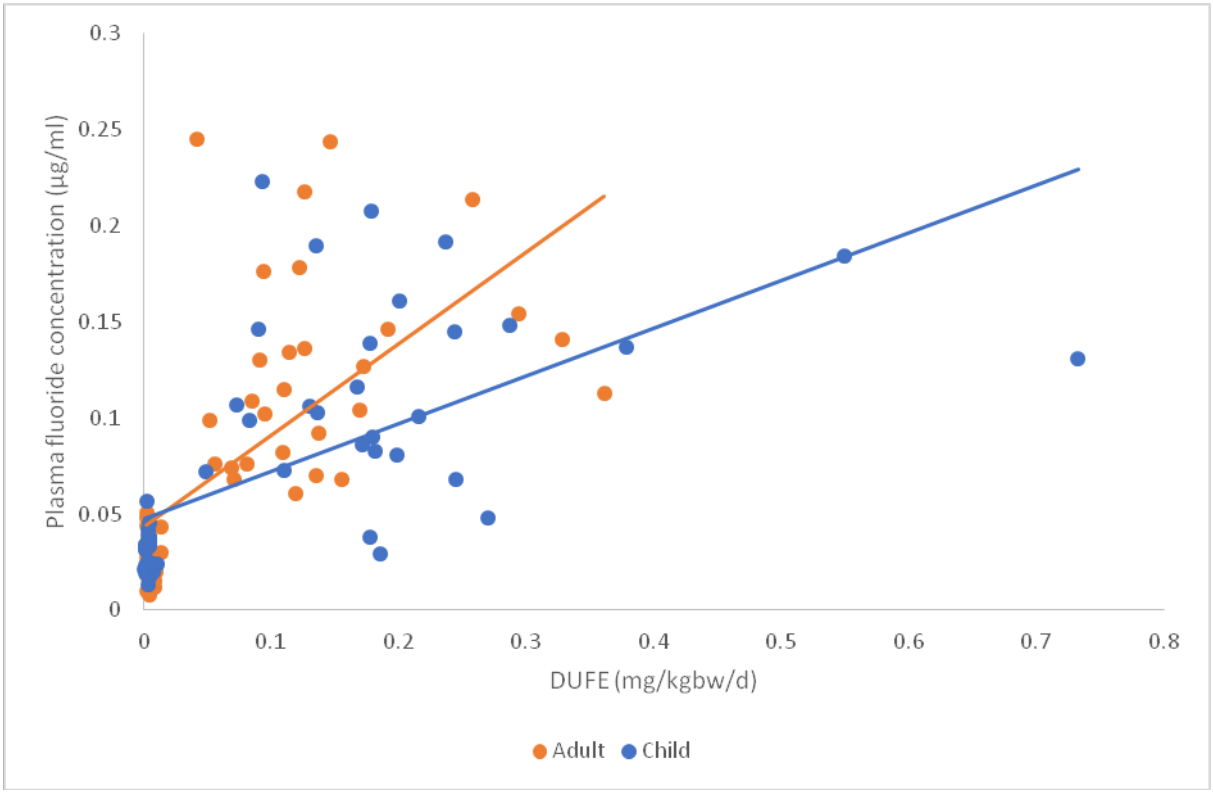

Figure 8.16 Relationship between plasma fluoride concentration and daily urinary fluoride excretion (DUFE). *[Plasma fluoride concentration (µg/ml) =0.048 + [0.24 x DUFE (mg/kgbw/d)] (children); Plasma fluoride concentration (µg/ml) =0.043 + [0.48 x DUFE (mg/kgbw/d)] (adults)*]

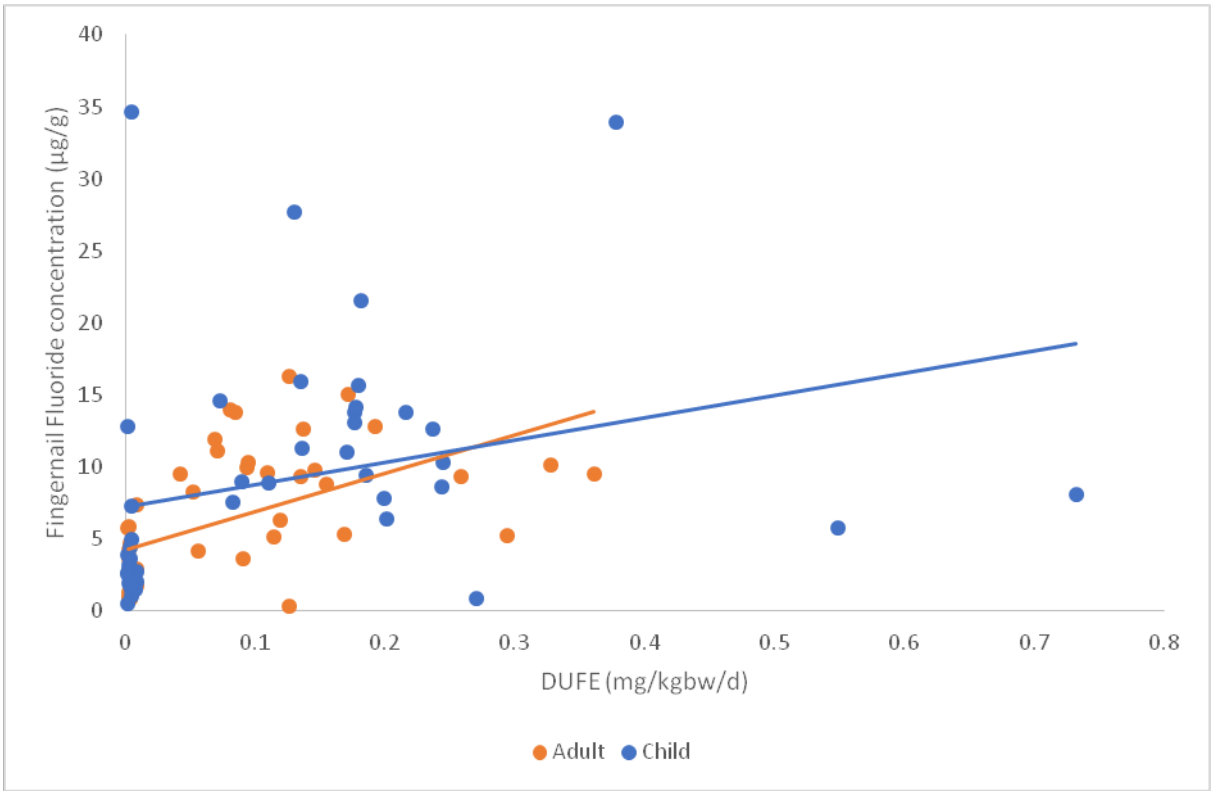

Figure 8.17 Relationship between fingernail fluoride concentration and daily urinary fluoride excretion (DUFE). *[Fingernail fluoride concentration (µg/g) =7.21 + [15.48 x DUFE (mg/kgbw/d)] (children); Fingernail fluoride concentration (µg/g) =4.21 + [26.58 x DUFE (mg/kgbw/d)] (adults)*]

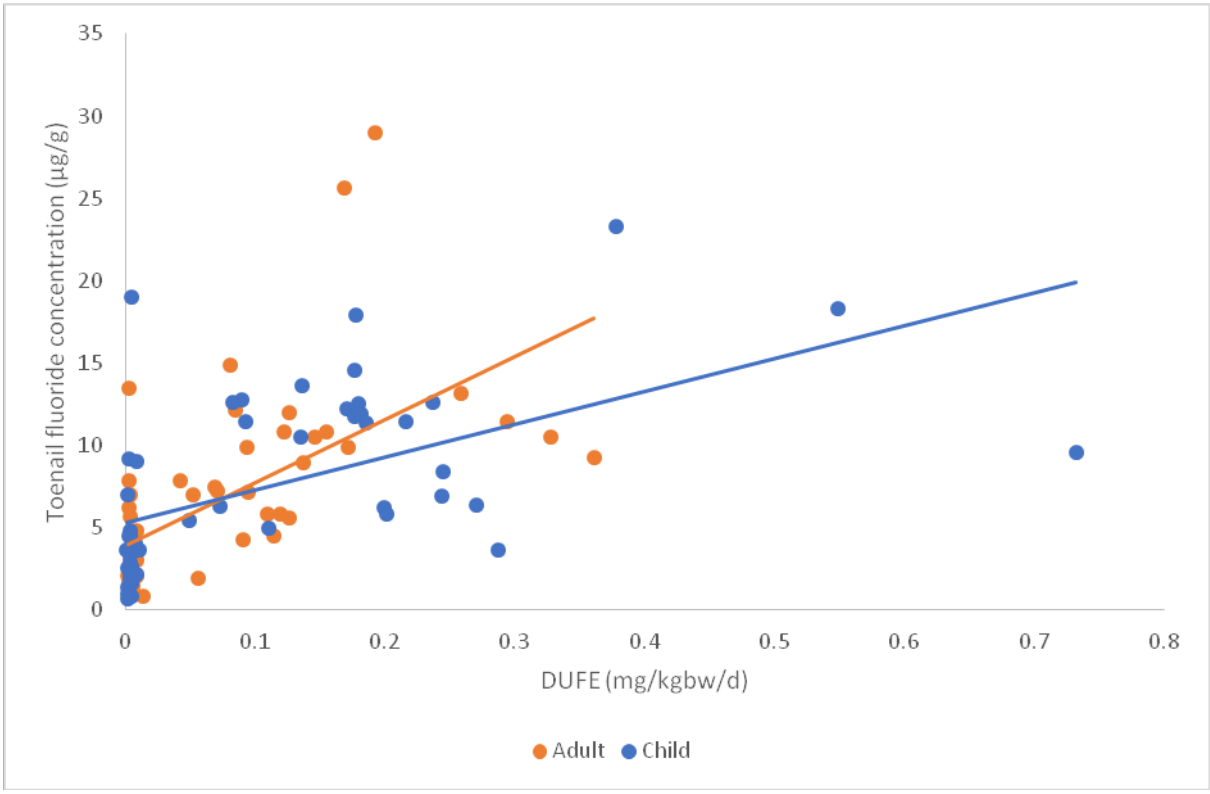

Figure 8.18 Relationship between toenail fluoride concentration and daily urinary fluoride excretion (DUFE). *[Toenail fluoride concentration (µg/g) =5.33 + [19.93 x DUFE (mg/kgbw/d)] (children); Toenail fluoride concentration (µg/ml) =3.95 + [38.08 x DUFE (mg/kgbw/d)] (adults)*]

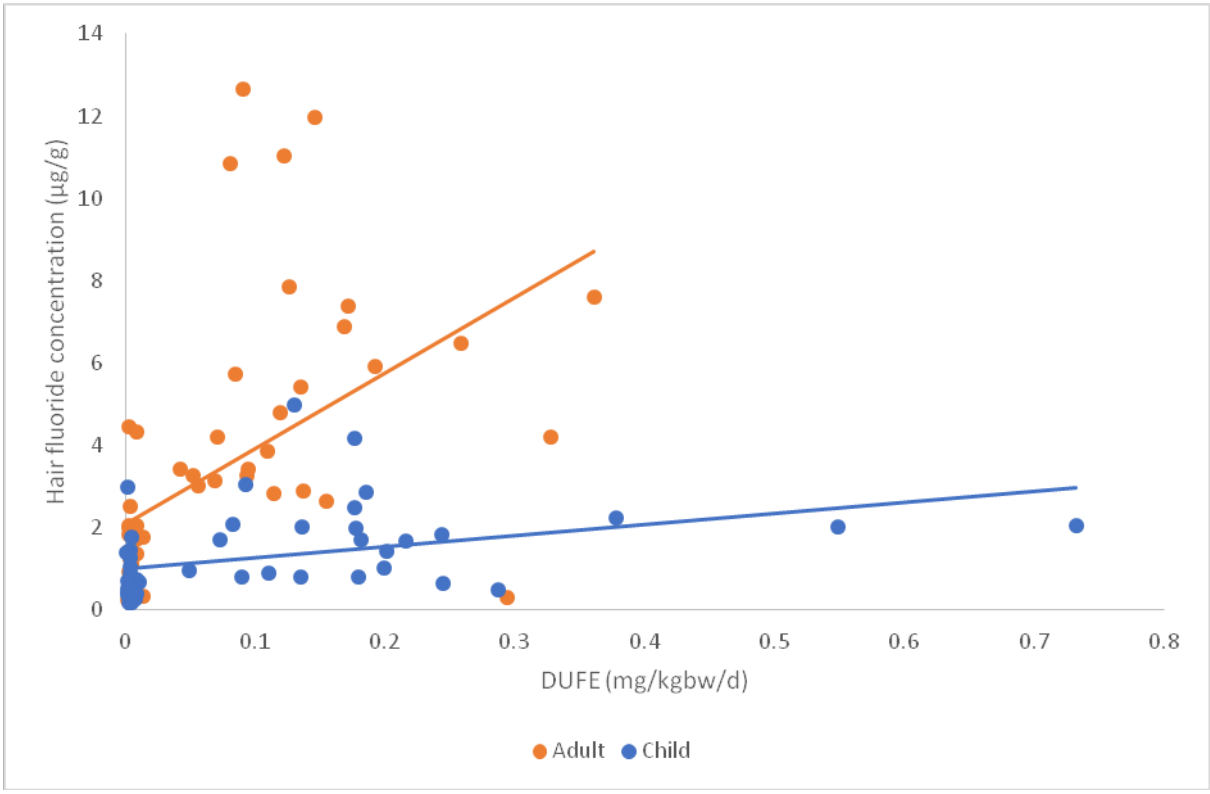

Figure 8.19 Relationship between hair fluoride concentration and daily urinary fluoride excretion (DUFE). *[Hair fluoride concentration (µg/g) =1.01 + [2.68 x DUFE (mg/kgbw/d)] (children); Hair fluoride concentration (µg/g) =2.09 + [18.33 x DUFE (mg/kgbw/d)] (adults)*]

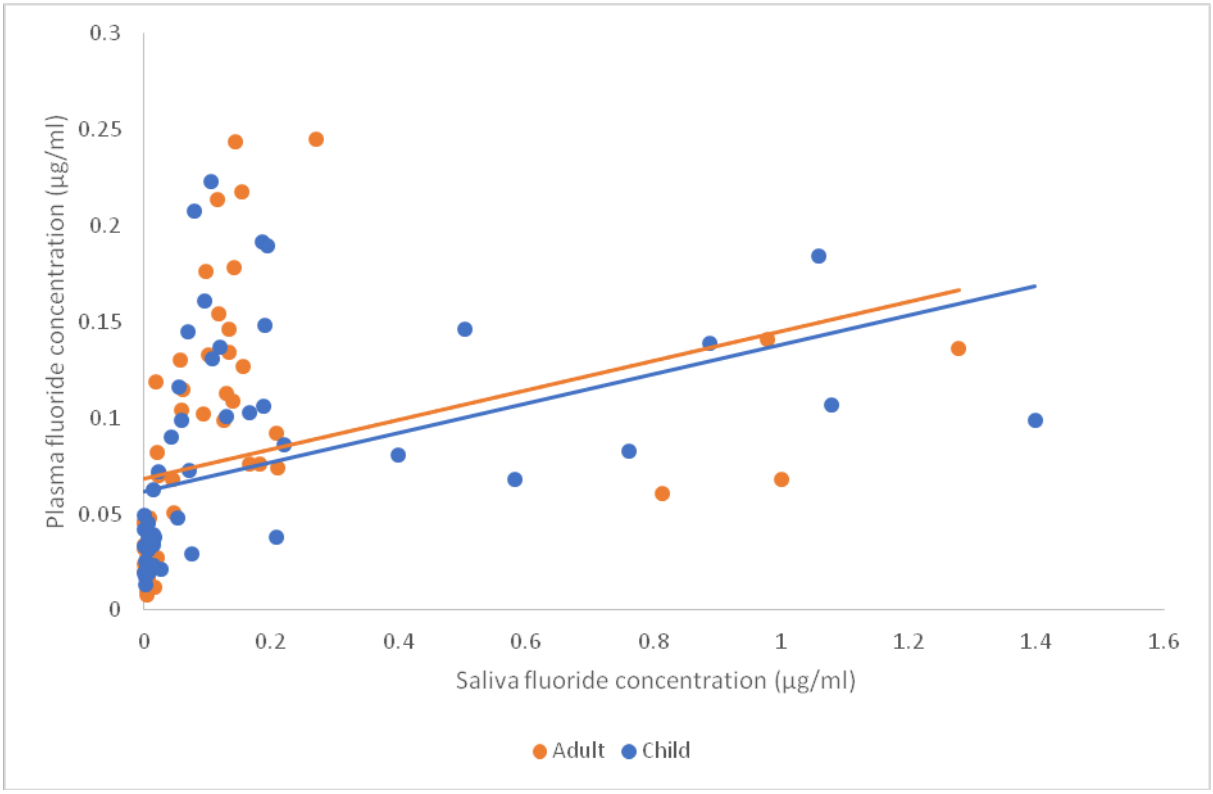

Figure 8.20 Relationship between plasma fluoride concentration and saliva fluoride concentration. *[Plasma fluoride concentration (µg/ml) =0.062 + [0.076 x Fluoride concentration in saliva (µg/ml)] (children); Plasma fluoride concentration (µg/ml) =0.068 + [0.077 x Fluoride concentration in saliva (µg/ml)] (adults)*].

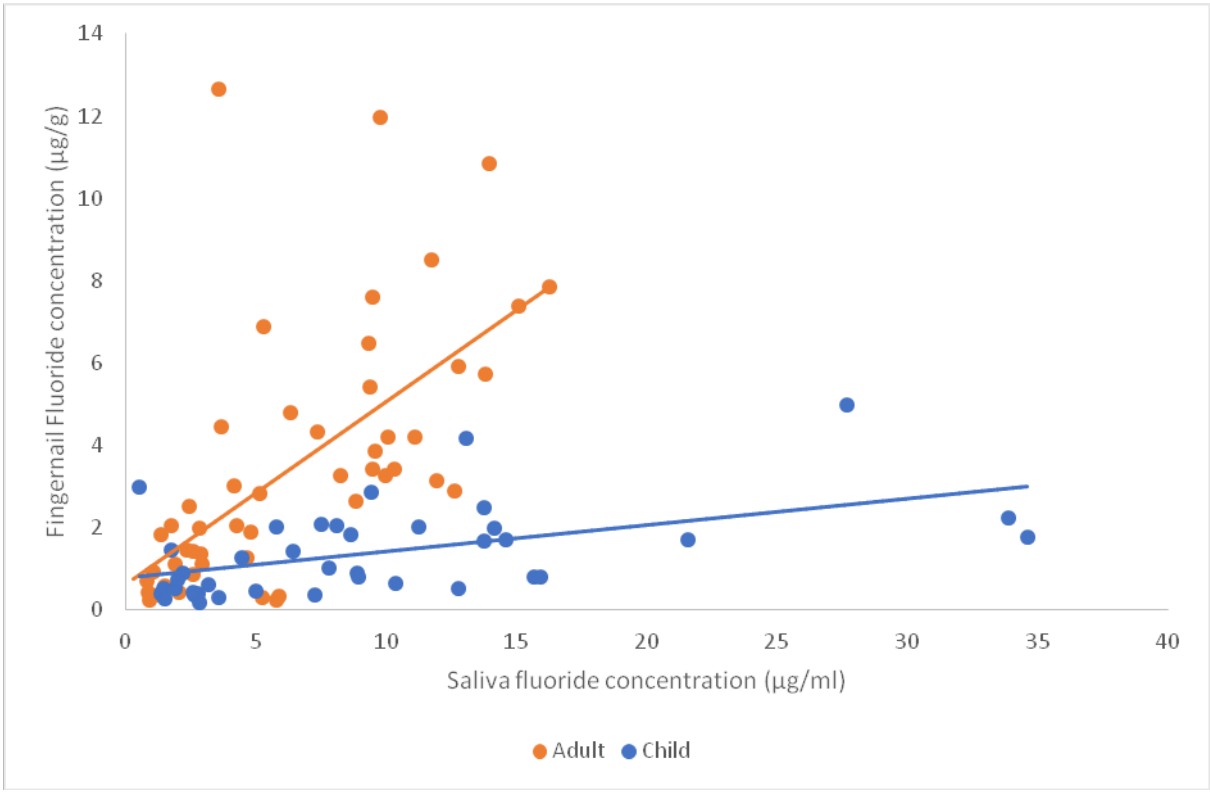

Figure 8.21 Relationship between fingernail fluoride concentration and fluoride concentration in saliva. *[Fingernail fluoride concentration (µg/g) =0.77 + [0.064 x Fluoride concentration in saliva (µg/ml)] (children); Fingernail fluoride concentration (µg/g) =0.64 + [0.44 x Fluoride concentration in saliva (µg/ml)] (adults)*].

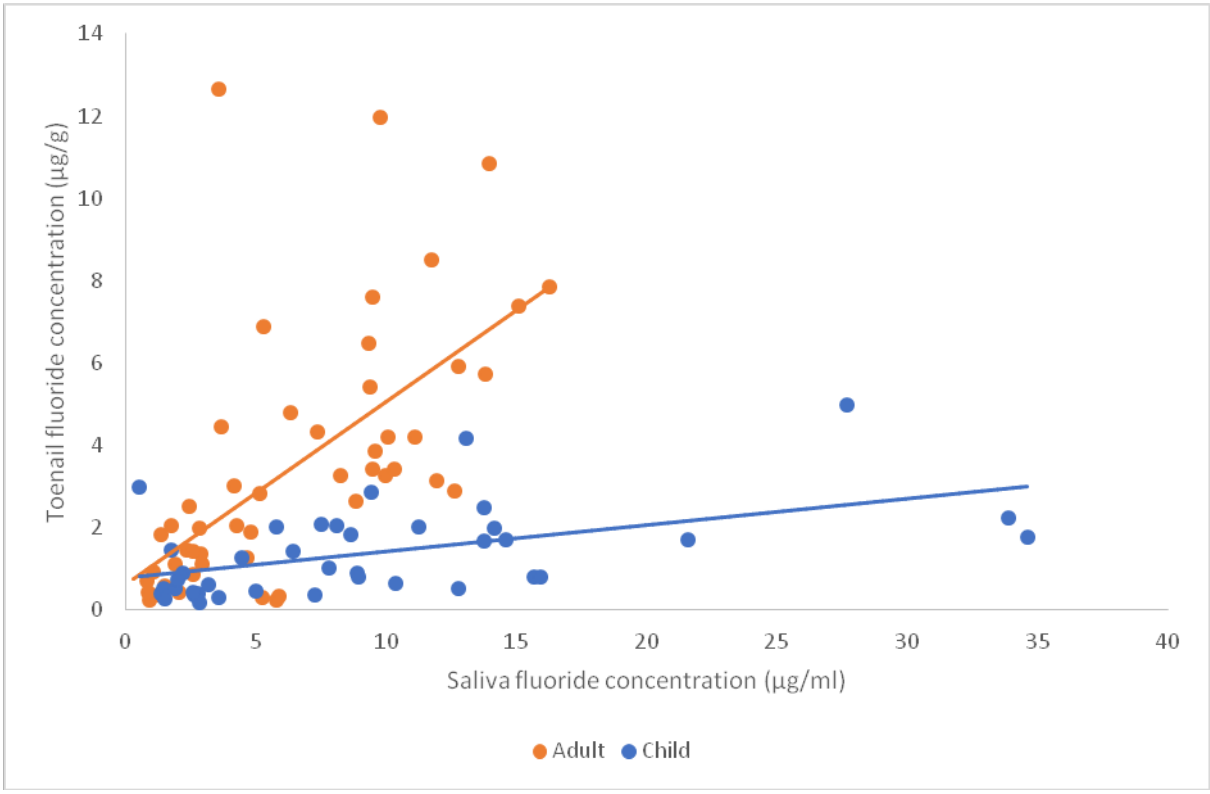

Figure 8.22 Relationship between toenail fluoride concentration and fluoride concentration in saliva. *[Toenail fluoride concentration (µg/g) =0.77 + [0.064 x Fluoride concentration in saliva (µg/ml)] (children); Plasma fluoride concentration (µg/g) =0.64 + [0.443 x Fluoride concentration in saliva (µg/ml)] (adults)*].

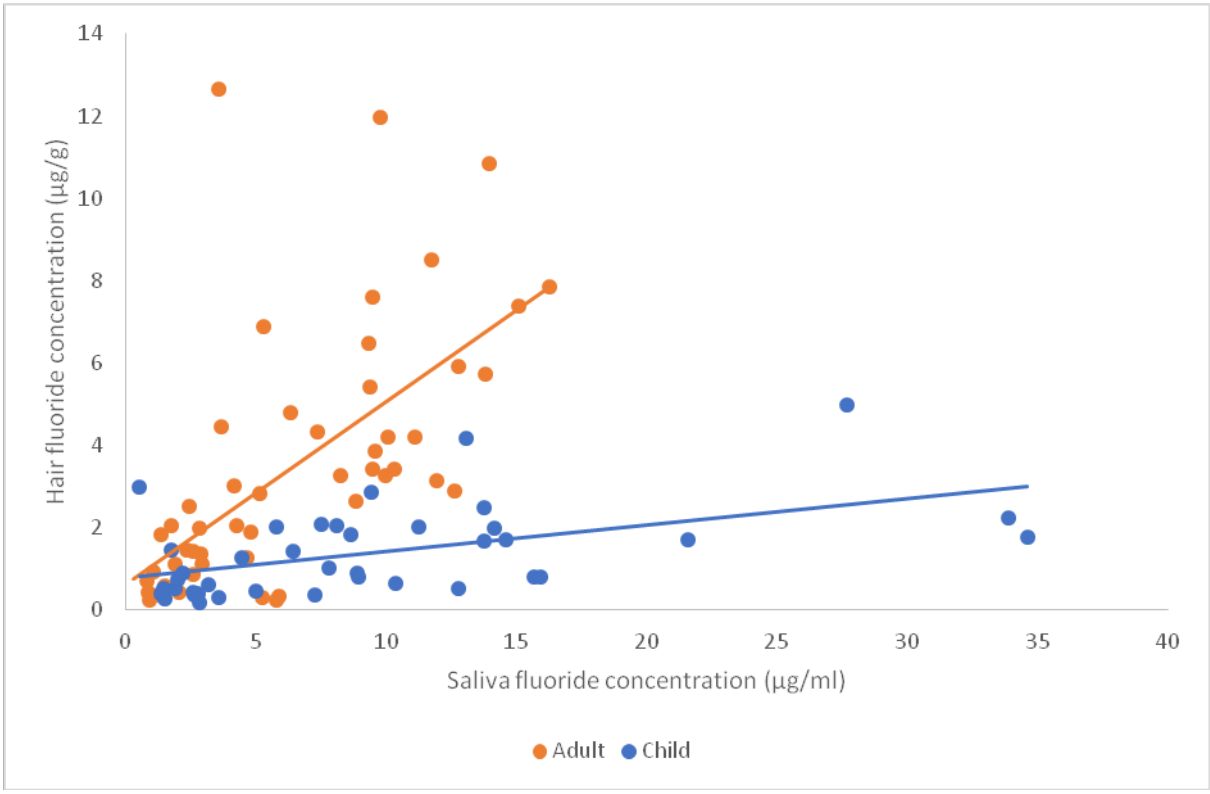

Figure 8.23 Relationship between hair fluoride concentration and fluoride concentration in saliva. *[Hair fluoride concentration (µg/g) =0.77 + [0.064 x Fluoride concentration in saliva (µg/ml)] (children); Hair fluoride concentration (µg/g) =0.64 + [0.44 x Fluoride concentration in saliva (µg/ml)] (adults)*].

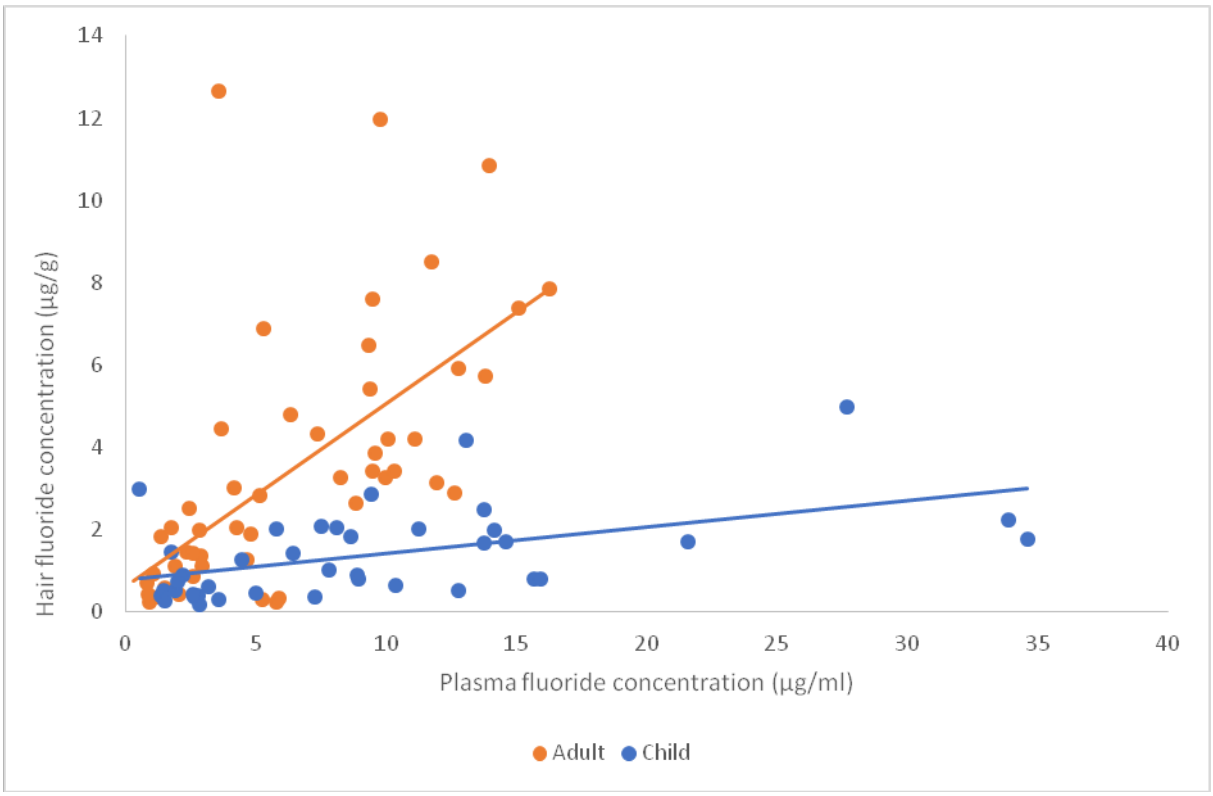

Figure 8.24 Relationship between hair fluoride concentration and plasma fluoride concentration. *[Hair fluoride concentration (µg/g) =0.77 + [0.064 x Plasma fluoride concentration (µg/ml)] (children); Hair fluoride concentration (µg/g) =0.64 + [0.44 x Plasma fluoride concentration (µg/ml)] (adults)*].

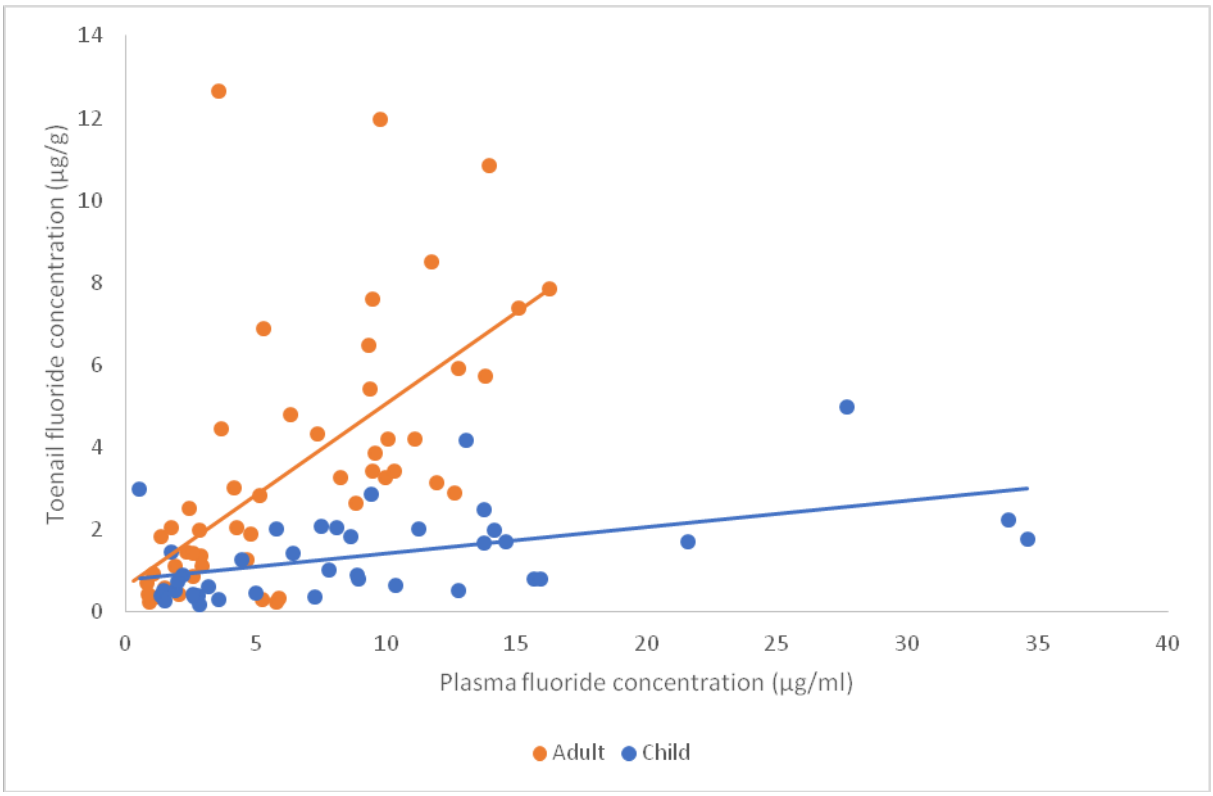

Figure 8.25 Relationship between toenail fluoride concentration and plasma fluoride concentration. *[Toenail fluoride concentration (µg/g) =0.77 + [0.064 x Plasma fluoride concentration (µg/ml)] (children); Toenail fluoride concentration (µg/g) =0.64 + [0.44 x Plasma fluoride concentration (µg/ml)] (adults)*].

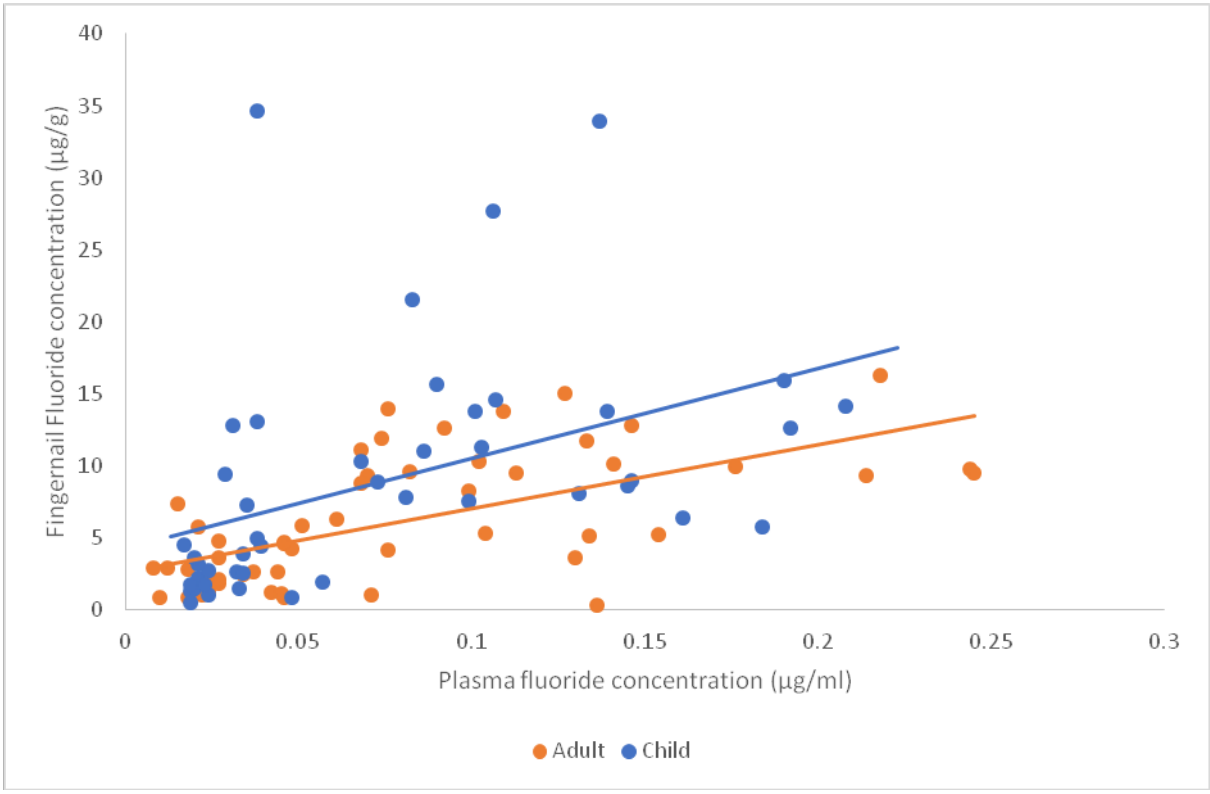

Figure 8.26 Relationship between fingernail fluoride concentration and plasma fluoride concentration. *[Fingernail fluoride concentration (µg/g) =4.33 + [62.25 x Plasma fluoride concentration (µg/ml)] (children); Fingernail fluoride concentration (µg/g) =2.66 + [44.14 x Plasma fluoride concentration (µg/ml)] (adults)*]**.**

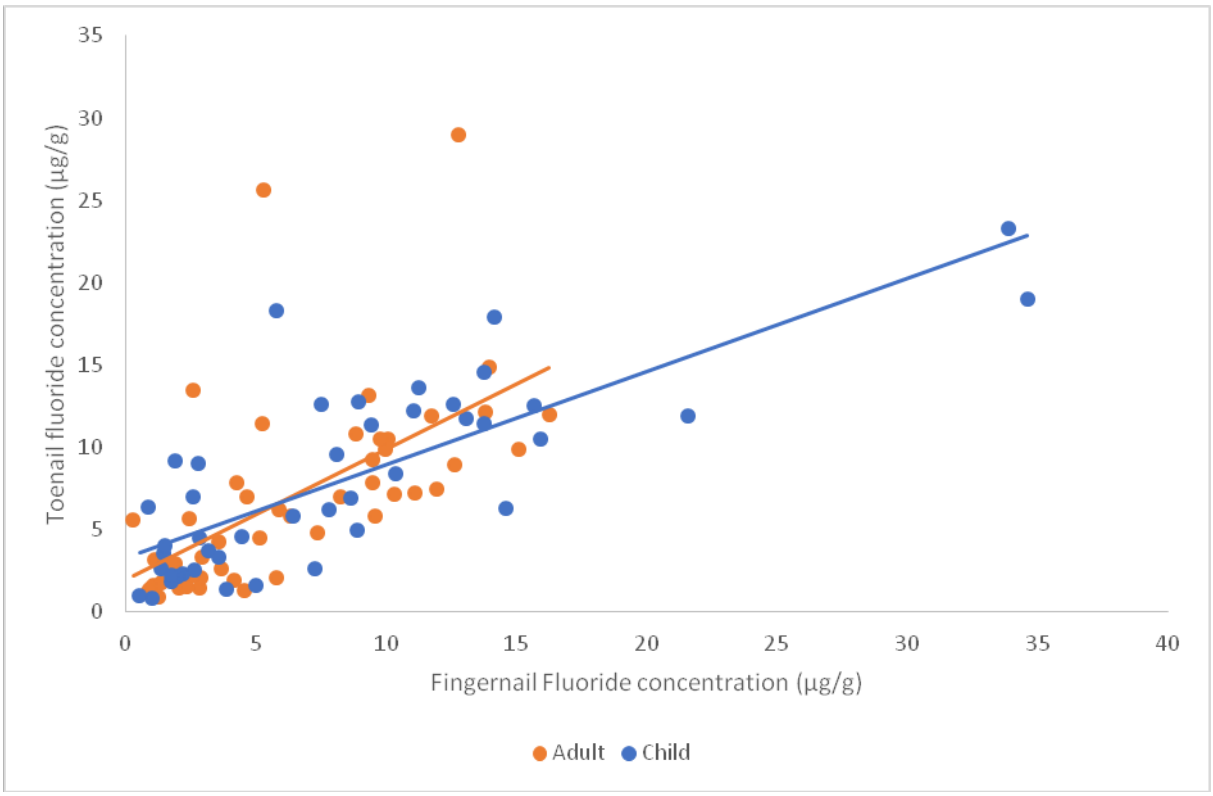

Figure 8.27 Relationship between toenail fluoride concentration and fingernail fluoride concentration. **[***Toenail fluoride concentration (µg/g) =3.34 + [0.56 x Fingernail fluoride concentration (µg/g)] (children); Toenail fluoride concentration (µg/g) =1.98 + [0.79 x Fingernail fluoride concentration (µg/g)] (adults)*].

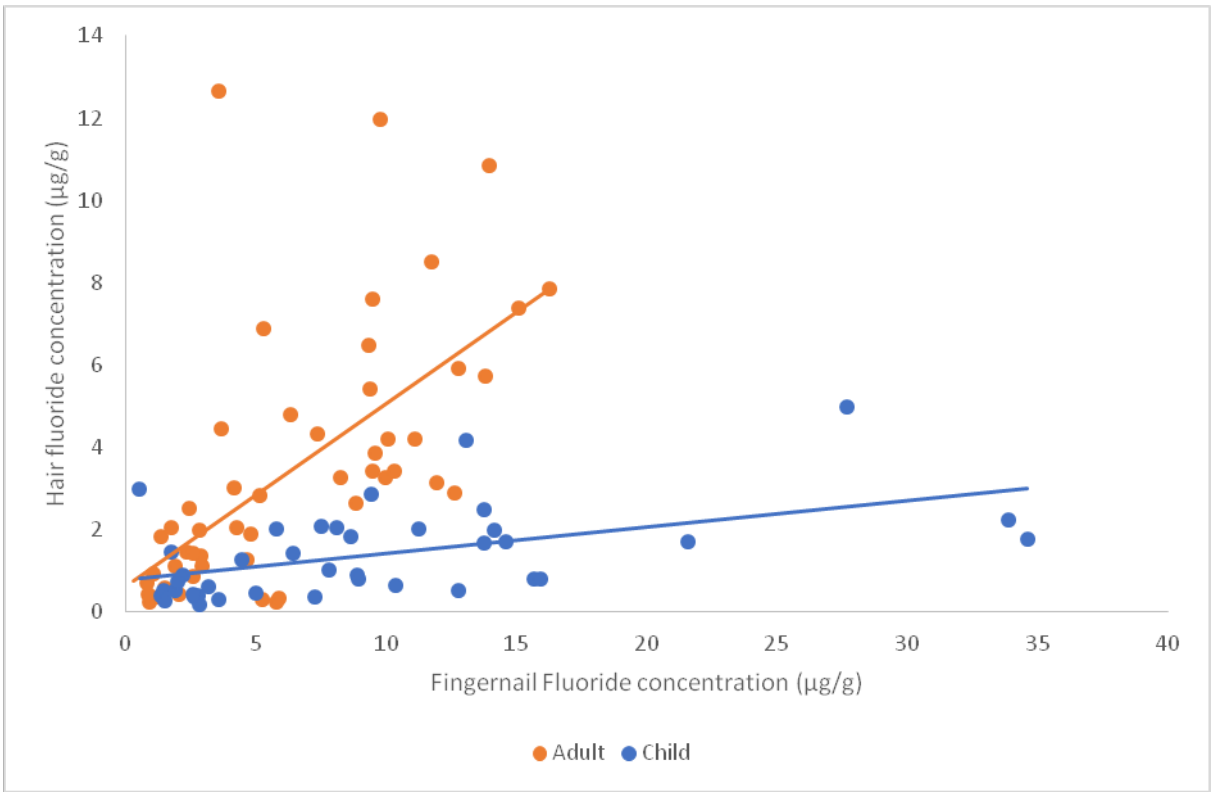

Figure 8.28 Relationship between hair fluoride concentration (µg/g) and fingernail fluoride concentration. *[Hair fluoride concentration (µg/g) =0.77 + [0.064 x Fingernail fluoride concentration (µg/g)] (children); Hair fluoride concentration (µg/g) =0.64 + [0.44 x Fingernail fluoride concentration (µg/g)] (adults)*].

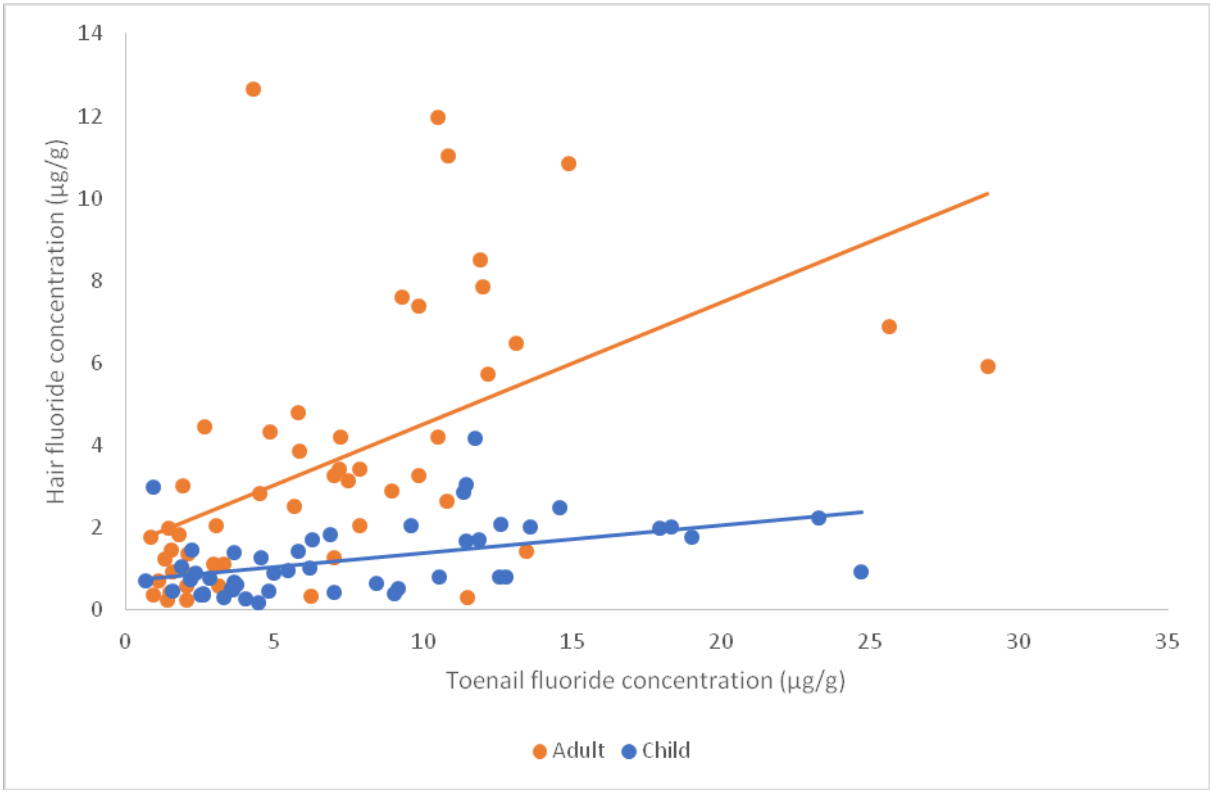

Figure 8.29 Relationship between hair fluoride concentration and toenail fluoride concentration. *[Hair fluoride concentration (µg/g) =0.71 + [0.068 x Toenail fluoride concentration (µg/g)] (children); Hair fluoride concentration (µg/g) =1.56 + [0.30 x Toenail fluoride concentration (µg/g)] (adults)*].

Summary

Table 8.26 presents the Pearson correlation coefficients and associated p values for the relationships between fluoride concentration in urine, saliva, plasma, hair, nail (fingernail and toenails) and daily urinary fluoride excretion in adults and children. There were positive correlations for all the biomarkers in both adults and children. Interestingly, both plots for plasma and saliva (Fig 8.20) appear to show a steep rise from the origin (0, 0) followed by a plateau after ca. F saliva = 0.15 µg/ml, rather than a continuous linear increase.

Table 8.26 Summary of relationships between various fluoride biomarkers and daily urinary fluoride excretion (mg/kgbw/d)

Fluoride Biomarker	Pearson correlation (p value)	
	Child	Adult
Saliva	0.424 (p=0.002)	0.460 (p<0.001)
Plasma	0.621 (p<0.001)	0.671 (p<0.001)
Toenail	0.542 (p<0.001)	0.599 (p<0.001)
Fingernail	0.401 (p=0.008)	0.551 (p<0.001)
Hair	0.383 (p=0.006)	0.520 (p<0.001)

8.16 Effect of age group and fluoride area on biomarkers of fluoride intake

A two-way analysis of variance was conducted on the influence of two independent variables (age group and fluoride water area) on dependent variables (urinary fluoride excretion, saliva fluoride retention, plasma fluoride retention, fingernail fluoride concentration, toenail fluoride concentration and hair fluoride concentration). Age group was children (4-5 years) and adults (> 20 years) and fluoride area was low fluoride and high fluoride. For all the biomarkers, the effect of age group and fluoride area is summarised in Table 8.27. For urinary fluoride excretion, the main effect of fluoride area yielded an F ratio = 119.0, $p < 0.001$, indicating a significant difference between high fluoride area (mean = 0.175, SD = 0.121) and low fluoride area (mean = 0.005, SD = 0.003). The main effect of age group yielded an F ratio = 4.932, $p < 0.05$, indicating that the effect of age group was significant, child (mean = 0.107, SD = 0.144) and adult (mean = 0.072, SD = 0.089). The interaction effect was also significant, F ratio = 5.223, $p < 0.05$.

For saliva fluoride, the main effect of fluoride area yielded an F ratio = 34.2, $p < 0.001$, indicating a significant difference between high fluoride area (mean = 0.272, SD = 0.344) and low fluoride area (mean = 0.009, SD = 0.008). The main effect of age group yielded an F ratio = 0.516, $p > 0.05$, indicating that the effect of age group was not significant, child (mean = 0.161, SD = 0.302) and adult (mean = 0.122, SD = 0.252). The interaction effect was not significant (F ratio = 0.522, $p > 0.05$).

For plasma fluoride, the main effect of fluoride area yielded an F ratio = 185.9, $p < 0.001$, indicating a significant difference between high fluoride area (mean = 0.121, SD = 0.051) and low fluoride area (mean = 0.030, SD = 0.013). The main effect of age group yielded an F ratio = 1.004, $p > 0.05$, indicating that the effect of age group was not significant, children (mean = 0.072, SD = 0.057) and adults (mean = 0.077, SD = 0.061). The interaction effect was not significant (F ratio = 0.458, $p > 0.05$).

For fingernail fluoride, the main effect of fluoride area yielded an F ratio = 87.2, $p < 0.001$, indicating a significant difference between high fluoride area (mean = 10.906, SD = 5.724) and low fluoride area (mean = 2.999, SD = 2.190). The main effect of age group yielded an F ratio = 4.439, $p < 0.05$, indicating that the effect of age group was significant, children (mean = 8.113, SD = 7.140) and adults (mean = 6.110, SD = 4.424). The interaction effect was not significant (F ratio = 2.566, $p > 0.05$).

For toenail fluoride, the main effect of fluoride area yielded an F ratio = 64.2, $p < 0.001$, indicating significant difference between high fluoride area (mean = 10.794, SD = 5.499) and low fluoride area (mean = 3.679, SD = 3.298). The main effect of age group yielded an F ratio = 1.219, $p > 0.05$, indicating that the effect of age group was not significant, children (mean = 7.796, SD = 5.829) and adults (mean = 6.812, SD = 5.727). The interaction effect was also not significant (F ratio = 0.048, $p > 0.05$).

For hair fluoride, the main effect of fluoride area yielded an F ratio = 61.5, $p < 0.001$, indicating significant difference between high fluoride area (mean = 3.356, SD = 3.084) and low fluoride area (mean = 1.067, SD = 0.921). The main effect of age group yielded an F ratio = 42.3, $p < 0.001$, indicating that the effect of age group was significant, children (mean = 1.266, SD = 1.025) and adults (mean = 3.452, SD = 3.180). The interaction effect was also significant at the 0.05 significant level (F ratio = 22.0, $p < 0.001$.

Table 8.27 Effect of age group and fluoride area on fluoride concentration in biomarkers

Biomarkers	Age group F ratio (p value)	Fluoride area F ratio (p value)	Interaction F ratio (p value)
Urinary fluoride excretion	4.932 ($p < 0.05$)	119.0 ($p < 0.001$)	5.223 ($p < 0.05$)
Saliva fluoride	0.516 ($p >$ ns)	34.2 ($p < 0.001$)	0.522 ($p >$ ns)
Plasma fluoride	1.004 ($p >$ ns)	185.9 ($p < 0.001$)	0.458 ($p >$ ns)
Fingernail fluoride	4.439 ($p < 0.05$)	87.2 ($p < 0.001$)	2.566 ($p >$ ns)
Toenail fluoride	1.219 ($p >$ ns)	64.2 ($p < 0.001$)	0.048 ($p >$ ns)
Hair fluoride	42.3 ($p < 0.001$)	61.5 ($p < 0.001$)	22.0 ($p < 0.001$)

Ns, not significant

8.17 DISCUSSION

8.17.1 Study location

The amount of fluoride in a biological marker is dependent on many factors e.g. physiological, environmental etc., associated with the use of such a marker as well as on fluoride exposure. A reliable biomarker of fluoride exposure should also be useful in predicting exposure to micro quantities of fluoride as well as in an endemic situation. Therefore, an endemic (high) fluoride water area and a low fluoride water area were selected to investigate the ability of the different biomarkers to effectively predict high and low fluoride exposure. Plateau state was selected due to its high variability of fluoride in drinking water between its different local government areas. Langtang local government area, within the Plateau state, had been reported to contain a very high concentration of fluoride in drinking water due to the geology of the region (Dibal *et al*., 2008) and some associated effects of fluoride, for example dental fluorosis and bowing of legs, had also been reported (Dibal *et al*., 2012a; Dibal *et al*., 2012b). The location of the study was selected following from the preliminary studies reported in Chapter 6 where samples of water were collected from the different local government areas of Plateau state. The final target areas were selected because of the large difference in water fluoride concentration between the two and because the people living there had virtually the same lifestyle and dietary habits.

8.17.2 Recruitment

Epidemiological studies where biological samples were collected have been few in Nigeria, and in some areas where these studies have been conducted there are conflicting issues

associated with religion, culture, payment, and fear of misuse of samples. Traditional rulers, usually referred to as Emir in the Northern part of Nigeria and Oba in the Western part of Nigeria, as well as their councils are very influential and respected and they play an essential role for any decision to be taken at the community level for projects as it affects their subjects. Research conducted in many developing countries requires some level of community involvement and consultation; consent from an individual may not be recognized without community consent. But the consent of the community is not a substitute for individual informed consent (Council of International Organisation of Medical Science, 2002). However, there might be conflict when an individual is willing to participate in research and there is no support from the community or when the community is involved but individuals are unwilling (Dave *et al*., 2007). Therefore, community consent as well as individual consent are a precondition for successful research in developing countries (Diallo *et al*., 2005. The meeting with the heads of each community for this project was very successful and the research received immense support from community leaders who provided the forum for communication with the community members from whom participants were selected. The chosen community cooperated well and was very helpful in ensuring compliance during the study period.

8.17.2.1 Response rate and gender

The present study was conducted after identifying the official and unofficial decision-making structure that exists within the community and positive consent from the community was obtained. Parents were contacted directly after their positive response had been received. Communication of the research also played a significant role in participation in the present study as an incentive was not offered to the participants. A total of 122 consented and took part in the study, only two participants (parent and child) dropped out of the study after prior acceptance and this was associated with miscommunication between the husband and wife regarding their participation; 120 completed the study. More women participated in the study than men in both fluoride areas due to their availability. In most developing countries, particularly in rural areas, women are more often at home to cater for the needs of the children as well as the husband. Most of the men are the main financial contributors to the family and are usually out of the house due to their occupation, whereas the occupation of most of the women in a rural community is farming which keeps them within reach. They are only unavailable when they are out at the market to sell their farm produce. In Nigeria, children start to attend school from age 3 years but the proportion of children who attend

school in urban areas is higher than in rural areas (NBS, 2010). The study was conducted in a rural area and some of the children aged 4-5 years living in the selected communities in the high fluoride area and low fluoride area did not go to school.

8.17.2.2 Sample collection

There was good compliance with the provision of the samples for all the participants who consented to the study. Participants who completed the study but could not provide some of the biomarkers was due to their unavailability or to the collection procedure. For nail and hair samples, which were the least provided, this was because participants had short nails or hair at the time of collection. Another reason could be attributed to a higher number of females compared to males who participated in the study. Females do not like to cut their nails and hair for personal reasons. However, in one case a nail sample was discarded from one of the female participants due to nail paint/varnish. In a study conducted in the Ethiopian rift valley, East Africa by Rango *et al*. (2017) missing samples were also reported from individuals not having sufficient sample for clipping or who had been unwilling to collect overnight urine. For this present study, blood and saliva were collected at a community centre whereas nail and hair samples were collected in the home of individual participants. This was particularly useful as a day was scheduled and agreed with all the participants when the nurse who collected the blood was to be present. Furthermore, for those who missed their appointment, another day was arranged with the community leader when the collection was done. There was a good line of communication between the community leader, the participants and the researcher. There was also a flexible approach by the researcher, who provided a research mobile phone contact number which participants could call whenever they had samples ready, particularly with nail and hair samples, and a time for collection was arranged with the researcher. Other studies have also successfully recruited all participants and collected samples by visiting their home for the collection of nail samples (Levy *et al*., 2004; Correa-Rodrigues *et al*., 2004).

8.17.2.3 Age and anthropometric data

Central incisors and first molars, which mineralize early, are highly susceptible to dental fluorosis if exposed to fluoride from the first year of life and to a lesser extent from the second year up to when the child is 6 years old (Bardsen and Bjorvatn, 1998). However, obtaining urine samples from this age group might be difficult due to their inability to control their bladder particularly during the night (Omid 2015). Exposure to fluoride early in life

can result in dental fluorosis in the permanent dentition (DenBesten and Li, 2012). In this regard, participants aged 4-5 years were selected for this study together with their parents aged ≥20 years to investigate age-related differences in the concentration of fluoride in the biomarkers. Children at this age can control their urination (Zohoori and Rugg-Gunn, 2000) and were able to participate in the research study with consent from their parents, who were also responsible for supervising their sample collection. It was pertinent to investigate the differences in growth rate/physiology etc. and therefore retention of fluoride in the bodies of adults due to issues of health and fluoride including hypotheroidism, osteosarcoma, thyroid cancer etc (MRC, 2002). as there are only a few studies on the fluoride concentration of biomarkers in adults exposed to either low fluoride- or high fluoride from all sources.

There were no differences in the weight and height as well as BMI of the children and adults living in the low- and high fluoride water areas. This is particularly useful as differences in the anthropometric characteristics might have influenced the interpretation of the data and therefore made it difficult to compare the two areas. The children's anthropometric values were within the WHO/NCHS reference standards for healthy weight (16.3-17.9 kg) and height (102.9-109.2 cm) (de Onis and Habicht, 1996) and in line with another study conducted among preschool children in Anambra state, Nigeria (Ilo *et al*., 2015). However, these values are lower than those obtained for British children 4-6 years old (Gregory *et al*., 2000) and 6-7 years old (Maguire *et al*., 2007) but higher than those obtained among 4 years old Iranian children living in low fluoride areas (Zohoori and Rugg-Gunn, 2000). The anthropometric characteristics of the adult participants in this study are similar to values obtained from a study conducted in Ota, Nigeria (Chinedu and Emiloju, 2014) but slightly higher than found among 18-24 years old adults in Ibadan, Nigeria (Sanusi, 2003). This might be due to the lower age group investigated in the latter study compared to the present study or time difference; the latter study was conducted more than 10 years ago. The adults in this study were slightly above the healthy weight range according to the BMI classification of the World Health Organisation (18.5-24.9 kg/m^2) (WHO, 1995).

8.17.3 Validation Checks

8.17.3 1 Fluoride analysis

In this study the two analytical methods, direct and indirect (HMDS-facilitated overnight diffusion method), were reliable. Typical variability for the analysis of samples between test and retest was negligible and similar for all types of biomarker. In urine and plasma, for

example, mean differences were only 0.006 and 0.007 μg/g, respectively. However, although the numerical reproducibilities for plasma and saliva were excellent, they represented relatively larger percentage errors compared to the values for the other types of sample because the fluoride concentrations in plasma and saliva were relatively low.

8.17.3.2 Completeness of urine samples

To ensure the completeness of the 24-hour urine sample collection, children who attended school opted to collect these samples on Saturdays when they were not in school to ensure full compliance. Adults who were meant to visit the market, cancelled their trip and remained at home. Others who were meant to be on the farm took their collection container with them. However, for the researcher to ensure the completeness of the urine sample collection, the urinary volume for each 24-hour sample was measured and the corresponding flow rate was calculated. According to WHO recommendations (WHO, 1999), urine samples with flow rates less than 5 ml/h or more than 160 ml/h were discarded in children and flow rates less than 9 ml/h or more than 300 ml/h were discarded in adults (WHO, 1999). It was assumed that these samples did not represent a 24-hour collection or they had been diluted with water and therefore did not represent the correct measure (Marthalar *et al*., 1995; Franco *et al*., 2005). In the present study, only one child sample and 2 adult samples from the low fluoride water area were rejected, whilst one adult sample from the high fluoride water area was rejected, for not meeting the validating criteria. This might be associated with some samples of urine not being collected in the container provided. Thus, a total of 112 samples from both fluoride areas, representing both adults and children, met the criteria recommended by WHO. Parents monitored their children to ensure that samples were collected in the storage containers as reflected in the high level of compliance.

8.17.4 Fluoride concentration of home drinking water, other drinks and food

8.17.4.1 Fluoride concentration in water

The study was conducted in low- and high fluoride areas whose source of drinking water was from boreholes with fluoride concentrations of 0.04 and 3.05 mg/l respectively. This was the water used by the community primarily for drinking, cooking and processing of foods which were sold in the community market or neighbouring markets. Low fluoride concentrations in drinking water have also been reported in western (El-Nadeef and Honkala) and southern parts of Nigeria (Apata *et al*., 2009; Ogwu *et al*., 2012). In the report of Apata *et al*. (2009) on the fluoride concentration of the different geopolitical zones of

Nigeria, 62% has drinking water fluoride ranging from 0.00 to 0.30 mg/l and 0.5% of the country is exposed to fluoride concentrations >2.50 mg/l. European countries and the UK (Ketley *et al*., 2004; Zohoori *et al*., 2006; Maguire *et al*., 2005, 2007) generally have a low fluoride concentration in drinking water whilst 55% of the US population with fluoride between 0.7 and 1.2 mg/l. High fluoride concentrations have been reported in some parts of Nigeria, particularly in the north, consisting of north-central, north-east and north-west areas with fluoride concentrations of 6.7 mg/l (Apata *et al*., 2009), 4 mg/l (Dibal and Lar, 2005) and 10.3 mg/l (Dibal *et al*., 2012), respectively. Despite the high level of fluoride in these areas, Waziri *et al*. (2012) reported a huge variation of fluoride between 0.02 mg/l and 2.07 mg/l in surface water collected from the north-eastern part of Nigeria, similar to what was obtained in the present study. The variation of fluoride in drinking water can be associated with the different rock compositions of the area: the associated fluoride-bearing minerals include: topaz ($Al_2SiO_4(FOH)_2$), calcium fluoride (CaF_2), fluorapatite ($Ca_5(PO4)_3F$), cryolite (Na_3AlF_6) and biotite (MgFeAlK, OH, F) (Lar *et al*., 2014). O'Mullane *et al*. (2016) reported that the general geology of an area is not an indicator of the fluoride concentration in groundwater but fluoride-containing formations at different depths. They concluded that there were significant variations in the distribution of rocks with fluoride that was readily leachable from their observation of wide differences in fluoride concentration in different wells in one village. Smedley *et al*. (2002) found no strong relationship between fluoride-bearing minerals and high concentrations of fluoride in groundwater in Ghana and Tanzania and concluded that geologic classification is not a perfect predictor of high fluoride water. The influence of depth on fluoride concentration in water was earlier reported by Boyle and Chignon (1995) in their study on the incidence of skeletal fluorosis in the Gaspe region of Quebec, Canada. However, more still needs to be known in this respect. A study conducted in Langtang local government area (LGA) of Plateau state Nigeria showed a very high variation in fluoride concentration between 0.12 and10.30 mg/l but the researchers could not associate the variation with depth as there were no differences in water collected in artisanal wells and boreholes, which were at different depths (Dibal *et al*., 2012). Langtang LGA is in an area of basement rock with groundwater found in three different aquifers: recently deposited alluvium found along river and stream channels, weathered overburden (soft overburden aquifer) and fractured crystalline aquifer. Dibal *et al*. (2012) also showed the distribution of fluoride based on the aquifers in the northern part of Nigeria, with fluoride concentrations from 0.03-10.30 mg/l in basement aquifers, 0.00-5.00 mg/l in sedimentary aquifers, 0.00-0.89 mg/l in young granite aquifers, and 0.00-0.78 mg/l in volcanic aquifers.

However, it was not clear which of the aquifers have the highest fluoride concentration due to the variation reported in each of the aquifer. Nor can the constituent rocks responsible for the fluoride concentrations in these areas be determined. Other factors have also been reported to influence fluoride concentration in water, including: water sources (e.g. spring, river etc.), solubility of the underlying rock (e.g. limestone) and seasonal variation. High fluoride between 2.0-20.9 mg/l has also been reported in boreholes in different parts of Kenya (Williamson, 1953; Manji and Nair, 1984; Kahama *et al*. 1997), Ghana and Tanzania (Smedley *et al*., 2002; Yoder *et al*., 1998; Vuhahula *et al*., 2010), India with 4.21-48.0 mg/l fluoride (Kotecha *et al*., 2012; Sexena and Sewak, 2015), and China (Quanyong, 2006).

In the present study, there might be a high risk for participants in the low fluoride areas to develop dental caries if they are not exposed to fluoride from other sources and their consumption of fluoride is not affected by environmental or genetic factors. Surprisingly, a mild form of fluorosis has been reported in these areas where fluoride concentration in the water is low (El-Nadeef and Honkala, 1998). In contrast, the children living in the high fluoride area are at risk of developing dental fluorosis because of high exposure to fluoride from drinking water and other food sources as well as fluoride supplements (Dibal *et al*., 2012; Yoder *et al*., 1998). This point is emphasised by the information summarised in table 8.28 and taken from a UK National Health Medical Research Council document (NHMRC, 1991).

Table 8.28 Exposure to fluoride and associated adverse effects (adapted from NHMRC, 1991)

Exposure to fluoride (mg/l drinking water)	Age	Effect
≥2	Child	Dental fluorosis
≥8	All ages	Skeletal fluorosis
≥50 (12 hours)	All ages	Gastroenteritis

8.17.4.2 Fluoride concentration in other drinks

In the present study, the food and drinks that were most frequently consumed were identified from the food frequency questionnaire and categorized as to whether they were prepared at home or prepared by the manufacturer. In the high fluoride area, fluoride concentration in the drinks was 2.4 and 5.2 mg/l in the guinea corn drink and a locally made alcoholic drink

called Burukutu, which is also made from cereal products including guinea corn, sorghum and millet but left about 3 days to ferment. The fluoride concentration of the grain (corn, wheat, rice) used to produce these drinks is relatively low (about 1 ppm), although, this value could vary depending on dust and soil (Waldbott, 1963). The high concentration of the drinks in the present study may be attributed to the concentration of fluoride in the water used for the preparation. However, the difference in the concentration of the different drinks might be associated with the amount of water used for preparing them or the sources of the cereals used. In the low fluoride area, a prepared maize drink had the lowest fluoride concentration, 0.031 mg/l. Among the drinks consumed in the low fluoride area, the fluoride concentration of tea made from tea bags was high (2.78 mg/l), similar to the tea drunk by children in North Carolina (2.56 mg/l) (Pang *et al*., 1992). The concentration of fluoride is not associated with the added milk, which usually contains <0.1 mg/l (Adair and Wei, 1978; Debeka *et al*., 1982), or the water used for preparation (0.04 mg/l fluoride) but can be attributed to the high natural fluoride content of the tea plant. The chocolate drink prepared with the same water has a fluoride concentration of 0.066 mg/l. The fluoride concentration of the carbonated drink (0.345 mg/l) is within the range described in previous literature (McClure, 1949; Pang *et al*., 1992; Zohoori and Maguire, 2014).

8.17.4.3 Fluoride concentration in food

The water used during preparation of food in the present study had a significant impact on the fluoride concentration between the low- and high fluoride area. There was a ten-fold increase in the fluoride concentration of beans and a 20-fold increase in guinea corn between the high- and low fluoride area but a 4-fold increase was found in bread. These differences can be attributed to the varying amount of water used in preparing the different foods.

The fluoride concentration in grains, except for the rice, in the present study ranged from 0.040 to 0.152 µg/g, whereas the concentration of rice was 1.597 µg/g. The concentration found in grain in the present studies is lower than what was found in studies reported in Sweden (1 µg/g) (Waldnott, 1963). The high fluoride content of the rice which is commonly consumed in the low fluoride area might be associated with a halo effect (Maguire and Zohoori, 2013), as it was not locally produced but usually imported from other countries and sold in Nigeria. The fluoride content of the vegetables; okra and kuka (made of dried baobab leaves) was 0.631 and 2.555 µg/g respectively. The fluoride content is associated with the concentration of fluoride in the soil where the vegetable is grown (Nommick, 1953). Wiatrowski *et al* (1975), in a study of dietary fluoride intake conducted among infants,

reported a range of 0.39-0.59 µg/g fluoride in most vegetables grown in the United States, but they reported a high fluoride concentration (2.02 µg/g) in creamed spinach. Baobab leaves might have a high fluoride content, which should be subject to further investigation. Where it was grown was not recorded in the present study. The fluoride content of potatoes reported in this study is 0.039 µg/g, similar to that found by Nommick (1953).

Fish is relatively high in fluoride, particularly when the fluoride content is measured with bones in it. The fluoride content of fish in this study is 1.449 µg/g, like the value reported (1.9 µg/g) by Chan *et al*. (2013) but lower than reported in previous literature, 40.0-84.47 µg/g (Lee and Wilson, 1935; Wei and Hattab, 1987) and 10 µg/g (Omid, 2015). The high fluoride content of fish reported by Omid (2015) was associated with the inclusion of bone when the sample was prepared for analysis but in the present study, where the fluoride content of fish is low, the analyses were conducted after the bones had been removed.

In the high fluoride area, the most common vegetables consumed were moringa and spinach with fluoride concentrations of 2.783 and 0.117 µg/g respectively. The fluoride concentration of spinach was low compared to the value reported by Wiatrowski *et al* (1975) and mentioned above. However, the latter value might be associated with the preparation of the spinach, particularly the cream. The fluoride concentration of meat including dog and pork was 0.312 and 0.998 µg/g respectively, without the inclusion of the bone during sample preparation. The value for pork is in the range 0.2-1.2 µg/g reported for fresh pork by Truhaut (1955).However, those authors found that salted pork had a higher fluoride concentration in a range of 1.1-3.3 µg/g, which is higher than that recorded in the present study. The fluoride content of pork was three times higher than that of dog meat. There has not been any reported range for dog meat because of the cultural differences related to the utilization of dog as food. In Nigeria, like in some other countries e.g. China, South Korea, and Vietnam, particularly in Plateau state where the study was conducted (high fluoride area), dog is the most consumed meat after pork.

8.17.5 Oral hygiene habits

8.17.5.1 Oral hygiene

There has been growing concern about a rise in the prevalence of dental fluorosis (Tabari *et al*., 2000; Pendrys, 2000) from the ingestion of fluoride from toothpaste, particularly in young children, following evidence of its bioavailability and systemic absorption close to 100% (Ekstrand *et al*., 1983). Evidence related to this risk factor has been subject to further

investigation following the variation in reports of additional risk in children exposed to fluoride toothpaste (Holm and Andersson, 1982; Osuji *et al*., 1988). However, studies have shown that children with good oral hygiene have significantly fewer carious lesions and filled proximal surfaces compared to children with poor oral hygiene (Henshaw and Adenubi, 1975; Mathiesen *et al*., 1996). In the present study, all the participants from the low fluoride area had good oral hygiene habits compared to those living in the high fluoride area, where 20% reported that they did not brush their teeth. This might be associated with their socio-economic status, which was not currently studied, or a lack of education as none of the children in the high fluoride area attended school. A study conducted in Lagos, Nigeria among 600 adolescents showed 96.3% brushed their teeth with toothpaste and 4% used a combined toothpaste and chewing stick regime (Umesi-Koleoso and Ayanbadejo *et al*., 2007). There might be excessive fluoride exposure for children living in a high fluoride area, who are already exposed to the high concentration of fluoride in the drinking water, where further use of fluoride toothpaste could elevate the level of fluorosis in their permanent incisors and first molars and on later erupting canines, premolars and second molars (Den Besten and Thariani, 1992; Levy, 2003). There are few reports on adult oral hygiene. In the present study, the percentages of parents who brushed their teeth and those who did not were the same as those of the children. This reveals that the attitude of parents to oral hygiene has a great influence on the children. Therefore, oral hygiene education needs to start from home or the invitation of parents to school for oral hygiene education of their children will provide better results.

8.17.5.2 Brushing frequency

It has been recommended by the European Academy for Paediatric Dentistry (2009) for children under age 6 months not to brush their teeth, children between 6 months and 2 years to brush twice daily with a pea-sized amount of toothpaste containing 500 µgF/g and children aged between 2-6 years to brush twice daily with a pea-sized quantity of toothpaste containing ≥1000 µgF/g In the UK, it has been recommended that children up to 3 years should brush twice/day with a smear of toothpaste containing at least 1000 µgF/g, while children aged 3-6 years are advised to use a pea-sized quantity of toothpaste containing 1350-1500 µgF/g (Department of Health/British Association for the Study of Community Dentistry, 2009). In the present study, 20% of children in the high F area did not brush at all and among the children who did brush their teeth in both fluoride areas, there was no difference in the number of times in a day, they brushed. Almost all respondents (96%)

brushed their teeth once per day, similar to the percentage reported by Umesi-Koleoso and Ayanbadejo *et al*. (2007) among adolescents in the southern part of Nigeria. This confirms the report from a literature review on oral health hygiene conducted by Folayan *et al*. (2014), which showed that the practice of brushing twice per day is low in Nigeria. In India, about 44% of children are reported to brush their teeth twice per day (Mascarenhas and Burt, 1998). In contrast, studies in the UK showed that between 69-76% of children aged between 4-6 years old brushed their teeth twice daily (Pendry *et al*., 2004; Zohoori *et al*., 2012). A higher percentage (64%) of twice daily brushing was also recorded in 3.5 years old children in the Netherlands (Cochran *et al*., 2004). The reason for this disparity could be associated with less awareness about the importance of oral hygiene for the teeth or socio-economic factors following from the percentage of people living on less than a dollar per day which is predominant in the rural area of Nigeria where the present study was conducted (Barau, 2009). However, the response from the adults made some of these factors evident, as 91.7% parents of children living in the high fluoride area brushed their teeth once whereas 29% of parents in the low F area brushed their teeth twice. This is associated with the fact that the level of education of parents who completed post-secondary education in the low fluoride area was more compared to the high fluoride area where most of the respondents had secondary education as the highest level of education. Considering the disparity that more parents in the low fluoride area brushed twice compared to their children, the findings show that less than 50% of the parents in the low fluoride area brushed their children's teeth whereas, in the high fluoride area, 97% of parents brushed the teeth of their child. This shows that, despite the awareness of some parents of the need to brush twice per day, because of lack of supervision of their child, the children brushed once. Although, for effective plaque removal, the amount of time spent brushing is more useful than the frequency of brushing (Honkala *et al*., 1986; Beals *et al*., 2000; Gallagher *et al*., 2009), the frequency of brushing is essential for the maximum benefit of fluoride in the mouth (topical effect and systemic effect) (Ekstrand and Ehrnebo, 1980; Ashley *et al*., 1999; 2001; Attin *et al*., 2005).

8.17.5.3 Type of toothpaste used

In the present study, all the respondents (in both low- and high fluoride areas) used toothpaste containing fluoride in the form of NaF, contrary to half that was obtained in a study conducted in the UK (Omid, 2015). The popular brand of toothpaste used by respondents in the low (83 %) and high (77 %) fluoride areas is Close-up toothpaste produced by Unilever Nigeria PLC, which has been in Nigeria for many years and readily

available in supermarkets in different flavours. However, in the current study, only 3% used the herbal Close-up type in the low fluoride area, whereas the remaining 84% used deep action Close-up with red-hot colour. A recent report on leading oral care brands in Nigeria showed that Close-up and Oral B were the top brands but consumers preferred Close-up because it is cheaper (Oriade, 2017). About 9% used Oral B in the low fluoride area which was introduced into Nigeria 5 years ago by Procter and Gamble (P&G). Surprisingly, 20% of the respondents in the high fluoride area used Macleans, which is the oldest toothpaste brand in Nigeria, whereas none used it in the low fluoride area. It was interesting to find that about 20% of the adults in the high fluoride area who participated in the study were aged between 48-56 years, whereas adults in the low fluoride area were ≤42 years old. Overall, 91% of the respondents used toothpaste containing 1450 µgF/g for the children which dropped to 87.3% for the adults. This might be associated with the use of chewing sticks by the parents, which is popular in the rural areas but undocumented in this study. The reason for the choice of toothpaste brand was not associated with the labelling, since virtually all children used the same toothpaste as their parents which contained a high fluoride concentration not recommended for children of that age group (DoH/BASCD, 2009; European Academy of Paediatric Dentistry, 2009). A recent study conducted by Ibiyemi (2016) at the University College Hospital, Department of Periodontology and Community Dentistry, Ibadan Nigeria in collaboration with Zohoori and Maguire from the Department of Child Dental Health, Newcastle University, showed that 4 Nigerian toothpastes out of 10, which were readily available for adults in both the UK and Nigeria, did not state the fluoride concentration on the label as it was done in the UK and only one had the information on brushing twice daily. In the present study, toothpaste labelled as containing 1450 µg/g measured 2100 µg/g after overnight diffusion with acid-HMDS except for one which was within the expected range and those labelled 1100 µg/g contained 1400 µg/g. The pastes may have been mis-stored and allowed to dry out during storage by commercial merchant of the products. The use of adult toothpaste in very young children observed in the present study is in line with what has been reported earlier. This can lead to a higher fluoride intake from toothpaste which might result in a slight increase of the risk of mild fluorosis in low caries communities (Tavener *et al*. 2006). A study conducted among 4-6 years old children in the UK (a low fluoride area) also showed 75% used toothpaste containing more than 1000 µgF/g and 39% used toothpaste containing 1350-1450 µgF/g (Zohoori *et al*., 2012). It is essential to also note that the children living in the high fluoride area are already exposed to a high fluoride concentration in their drinking water thereby putting them at greater risk.

8.17.6 Fluoride intake from diet

8.17.6.1 Weight of food, drinks and water

Of interest, children in this study (in both high- and low fluoride areas) consumed a higher amount of food (1783 and 1525 g/day respectively) than 4-6 years old children (734-839 g/day) in the UK (Omid *et al*., 2016) and similar-aged children (558 g/day) living in Mexico City (Martinez-Mier *et al*., 2003).

Water consumption contributed immensely to the overall liquid consumed in both fluoride areas compared to the other drinks, as it is available without any cost except for bottled or sachet water. In the present study, the differences in consumption and weight are not statistically significant in the fluoride areas. It can be inferred that their dietary habits are similar.

The amount of beverages consumed in this study was low in both low and high fluoride areas (188 and 250 g/day respectively) compared to corresponding amounts recorded among Mexican children (701 g/day) (Martinez-Mier *et al*., 2003), Japanese children 2-8 years old (596-711 g/day) (Nohno *et al*., 2006), 4-6 years old children living in North Carolina, USA (1048 ml/day) (Pang *et al*., 1992) and 4-6 years old UK children (293-329 g/day) (Omid *et al*., 2016). There has been a shift in most developed countries from drinking water to the consumption of beverages, which might not be possible in most developing countries due to socioeconomic factors. Consumption of bottled mineral water is high compared to beverage intake in Brazil, which is a developing country due to its low Gross Domestic Product (GDP) per capita: for example, a 30% consumption of bottled mineral water was reported in Bauru (Ramires *et al*., 2004). Long ago, McClure (1943) suggested that 4-6 years old children should drink 1600 g water per day. Water consumption in the present study was low (420-457 g/day) compared to that observed among 4 years old children in Iran (1136 g/day) and 4-6 years old children in the USA (1520 g/day) but high compared to that observed among Peruvian children (271-299 g/day) (Rodrigues *et al*., 2009) and among UK children (139-204 g/day) (Omid *et al*., 2016). These variations might be associated with the different dietary habits of these children, differences in temperature (Zohoori and Rugg-Gunn, 2000) and to the methods of collection of the data. Duplicate diet collection has been recognised as the gold standard method (Zohoori *et al*., 2013; Nohno *et al*., 2006) but, due to its cost, a much cheaper method – the food frequency questionnaire (Miazira *et al*., 2009) - was adopted in the present study. This technique is an efficient, easy-to-use way to assess the

usual diet of a population (Slater *et al*., 2003). However, there might be chance of over- or underestimation of serving size, as this is dependent on the response of the parents of the children. The differences in environmental parameters within the countries might be another limitation to comparing the data.

There is limited information on the amount of food consumed by adults. In the present study, the consumption of food, beverages and water in the low fluoride area was 2749, 411 and 829 g/day and in the high fluoride area 3036, 650 and 704 g/day, respectively. The consumption of adults is double the amount that was consumed by the children. A study reported that the choice of portion size can vary with age and socioeconomic background (Benton, 2015) as well as a tendency to eat more when more food is available due to the large portion size (Wansink and Park, 1996; Rolls *et al*., 2002, 2004, 2007). In the present study, adults were served with larger plate sizes compared to the children which might have led to their higher food consumption.

8.17.6.2 Dietary sources of fluoride intake

Data on dietary fluoride intake from different sources was generated through a food frequency questionnaire (FFQ) completed by the parents and on behalf of their children. The FFQ was carefully designed to cover the food items consumed by the respondents living in the areas studied. It included reference portions associated with the sizes of eating plates and drinking cups provided by the people living in the communities prior to the commencement of the study. To ensure accuracy, the participants were shown the commonly used plates and cups (provided by members of the community) which had being labelled appropriately for them to estimate their usual consumption/portion sizes whether full, 1/2, ¾ and ¼. (See figure 8.2) This method (FFQ) in the present study was a slight modification from other studies which used picture charts to describe the different portion sizes of the amount of foods and drinks consumed by the participants (Levy *et al*., 2001; Broffitt *et al*., 2004; Miziara *et al*., 2009). These picture charts do not seem to work in the present study due to the different types of plates and cup sizes used in Nigerian communities and particularly in the rural areas where the present study was conducted. The FFQ was easily administered by trained persons, who communicated in the language of the people (see figure 8.1), and was cheaper than the commonly used duplicate method where participants keep a portion of all foods consumed and which involves a certain cost. In the latter case, a participant might, therefore, be tempted not to eat in the usual way to save some money and not keep a duplicate

of certain food. To prevent over- or under reporting, which is associated with the use of FFQs, the interviewer told the participants that the FFQ was strictly for the present research and not for government allocation or for some other purpose. In addition, they were attended to separately for confidentiality and elimination of fear associated with their neighbours getting knowledge of how much they ate. The FFQ contained 44 food items generally consumed in both areas. This number was lower than the 70 food items reported in a Brazilian study (Miziara *et al*., 2009). However, to avoid possible bias in food reporting, there were open-ended food groups like vegetables and fruits, since the choice of vegetables and fruits depended on what was readily available in the community.

Figure 8.30 Interviewer collecting information for the FFQ

Figure 8.31 Examples of plates and cups with labels for identification

The present study is the first report comparing retention of fluoride in the bodies of young children and adults living in a low fluoride area and an endemic fluoride area, and the first reported in Nigeria for adults' ≥20 years old. There was a significant difference in the intake

from the two main dietary sources between the low fluoride area and the high fluoride area. This could be associated with the fluoride concentration of drinking water, which is also used in the preparation of the diets, as the populations of the two fluoride areas have the same dietary habits. Furthermore, no significant differences were reported in their levels of consumption. For all children, the mean intake of fluoride from food was 0.726 mg/d and 1.461 mg/d and, when adjusted for body weight, 0.045 and 0.100 mg/kgbw/d in low and high fluoride area respectively. The reported fluoride intake in both fluoride areas in the present study is high compared to 0.225-0.267 mg/d among 4-6 year olds in the UK (Omid *et al*., 2016), 0.209 mg/d obtained among 6-7 years old children receiving optimally fluoridated water (0.82 mg/l) in the north east of England (Zohoori *et al*., 2006), 0.130 mg/d among 2-6 years old Brazilian children (Miziara *et al*., 2009), 0.130 mg/d among 15-30 months old children (Martinez-Mier *et al*., 2003) and 0.146 mg/d among 16-40 months old children (Rojas-Sanchaz *et al*., 1999) in the USA, 0.521-0.588 mg/d among 15-36 months old Mexican children exposed to fluoride from salt (1.9 g/d) (Martinez-Mier *et al*., 2003). It is difficult to compare these published results with the present findings due to differences in dietary assessment methods and in some cases age differences. However, the value obtained in the low fluoride area when adjusted by body weight is similar to that determined using the duplicate method among 22-35 months old children (≤0.04 mg/kgbw/day) in four Columbian cities with different socioeconomic status exposed to different fluoride concentrations in drinking water (0.006-0.009 mg/l) (Franco *et al*., 2005). It is obvious that none of the above previously published values are comparable to the value obtained in the present high fluoride area because of the high fluoride concentration in the drinking water which was also used in preparing the food.

Fluoride intake from drinks including water was 0.259 and 2.277 mg/d and when adjusted by weight 0.015 and 0.166 mg/kgbw/day in the low- and high fluoride area respectively. The value obtained from the low fluoride area is similar to that measured by a 3-day diary fluoride method for 4-6 years old children in the UK (0.266 mg/d) drinking fluoridated water (0.9 mg/l) (Omid *et al*., 2015). This is interesting since the children in the UK were exposed to a higher fluoride concentration in their drinking water. However, the similarity is due to intake of fluoride from other drinks by the Nigerian children, which amounted to 0.233 mg/d associated with consumption from tea, the highest contributor to the overall dietary intake in the low fluoride area (25.7 %, see Table 8.11), whereas in the UK, fluoride intake from water and other drinks was 0.132 and 0.134 mg/d respectively. The F intake value is lower

than those reported among US children, 0.422 mg/d (Martinez-Mier *et al*., 2008), 0.36-0.60 mg/d (Pang *et al*., 1992) and 0.396 mg/d (Rojas-Sanchez *et al*., 1999)), among 6-7 years old children in an optimally fluoridated area of north-east England, 0.382 mg/d but the value was slightly higher than the sub-optimally fluoridated area (0.210 mg/d) (Zohoori *et al*., 2006). A study conducted among 2-6 years old children in Brazil, reported a higher value (0.348 mg/d) (Miziara *et al*., 2009). However, for children living in Brazil when the fluoride intake from drinks was adjusted by body weight, the values were close, 0.020 mg/kgbw/d compared to 0.015 mg/kgbw/d observed in the low fluoride area of the present study, but higher than observed among Columbian children (0.004-0.005 mg/kgbw/d) (Franco *et al*., 2005). The small differences among the Brazilian and Nigerian children could be associated with drinking water fluoride concentration, whereas for the Columbian children it was not confirmed if the water was included in the measured beverage intake. Interestingly, the dietary estimates conducted in Brazil and Nigeria were assessed through FFQs. The fluoride intake from drinks (low fluoride area) in the present study was also in the range 0.18-0.32 mg/d reported among 2-8 years old children in a moderate fluoride area (0.555 mg/l) of Japan (Nohno *et al*., 2006). This similarity cannot be associated with the fluoride concentration in drinking water or the amounts of beverages consumed but might be associated with the fluoride concentrations of the beverages, which were not reported in the Japanese study. In the present study, fluoride intake from drinks in the high fluoride area was high compared to what was reported in previous studies where intake from drinks was identified.

Fluoride intake from drinking water in the present study was very low (0.036 mg/d) among children in the low fluoride area compared to that of the children in the high fluoride area (1.184 mg/d) due to the fluoride concentration in drinking water which was about 80 times different. The fluoride intake in the low fluoride area is low compared to that of children in Brazil (0.188 mg/d) (Miziara *et al*., 2009), among 4-6 years old children (0.132 mg/d) (Omid *et al*., 2015) and 6-7 years old children living in optimally fluoridated area (0.083 mg/d) and sub-optimally fluoridated area (0.081 mg/d) (Zohoori *et al*., 2006). This is associated with the low fluoride concentration in drinking water reported in the present study (0.04 mgF/l).

In adults, there was a significant difference in fluoride intake from the two major dietary sources in both the high- and low fluoride areas. Fluoride intake from food was 1.367 and 2.582 mg/d, and when adjusted for body weight 0.021 and 0.041 mg/kgbw/d, in the low- and high fluoride areas, respectively. The fluoride intake from food in the high fluoride area

is double that observed in the low fluoride area. Surprisingly, the fluoride concentration in the drinking water from the high fluoride area was 75 times higher than in that of the low fluoride area. Nevertheless, the fluoride intake found in the low fluoride area in the present study is high compared with fluoride intake from food among North American adults (0.3-0.5 mg/d). The relatively high intake of fluoride from food in the low fluoride area reflects the fact that the food consumed is not produced in the area and a resultant halo effect takes place. The major contributor to this relatively high fluoride intake in the low fluoride area was intake from rice (21.6 %) and a drink made from milk and processed maize (34.3 %), Table 8.13. The rice is not produced in the area and brought into the community for consumption. The fluoride concentration in milk is generally low, but the milk measured in this study is usually mixed with processed maize. It is not known if the water used in the processing of the maize had a high fluoride content. In the high fluoride area, the groundwater is usually used for cooking, therefore this water will undoubtedly contribute to the fluoride concentration of the food. Also, some of this food is grown in the area, although in the present study the source of the food was not specifically determined.

Fluoride intake from drinks (including water) in the low- and high fluoride water areas, respectively, was 0.537 and 4.933 mg/d and, when adjusted for body weight, 0.008 and 0.078 mg/kgbw/d. In the low fluoride area, tea (from tea bags) contributed 26% to the total dietary fluoride intake (Table 8.13). Tea accumulates fluoride over time and the older leaves which are used in most economic brands may contain high fluoride (Shu *et al*., 2013). There is a chance that these are the brands purchased in the rural areas studied here. In the low fluoride water area, the volume of tea drunk by adults was 510 ml/d, which is similar to the value recorded in the UK national diet (540 ml/d) by a nutrition survey in adults aged 19-64 years old (Handerson *et al*., 2002). In the high fluoride area, we could associate the high fluoride intake from drinks to the high fluoride concentration in the water the people drank from boreholes, which is the major source of drinking water (the volume of borehole water consumed was 1437 ml/d). However, the present study also showed that an alcoholic drink contributed about 40% to the total daily fluoride intake (the volume drunk by adults was 5459 ml/d).

Fluoride intake by adults from water in the present study was 0.082 and 1.867 mg/d and from other drinks was 0.454 and 3.065 mg/d in the high- and low fluoride areas, respectively. A recent study conducted in adults aged 10-50 years old living in the Ethiopian Rift Valley with widely varying groundwater fluoride concentration (0.6-15 mg/l) showed the fluoride

intake from water to be 9.8 mg/d (Rango *et al*., 2017), which is high compared to the value found among adults living in the high fluoride area in the present study. This difference might be associated with the difference in the fluoride concentration of the water. Interestingly, the amount of groundwater consumed by the Ethiopians was similar (1500 ml/d) to the volume of groundwater consumed in the high fluoride area (1437 ml/d) of the present study. The fluoride intake from water in the high fluoride area was similar to the value (1.612 mg/d) found among 20-40 years old females in Chile (Villa *et al*., 2008) who drank about 2000 ml/d water.

8.17.6.3 Total daily dietary fluoride intake (TDDFI)

There was a significant difference in the TDDFI among children in the present study between the low- (0.985 mg/d) and high (3.738 mg/d) fluoride water areas. The TDDFI reported in the low fluoride area is high compared to what was recorded in previous studies: among 3-4 years old Hungarian children (0.22-0.72 mg/d) (Schamschula *et al*. 1988), 0.15 mg/l among 3-4 year olds in New Zealand (Guha-Chowdhury *et al*., 1996), 0.28 mg/d among 1-6 years old children in Japan (Kimura *et al*., 2001), 0.208 mg/d among 3-6 years old German children (Haftenberger *et al*., 2001), 0.602 mg/d among 1.3-6 years old Columbian children (Franco *et al*., 2009), 0.478 mg/d among 2-6 years old Brazilian children (Miziara *et al*., 2009) and 0.188-0.583 mg/d among 6-7 years old children living in the UK (Maguire *et al*., 2007; Zohoori *et al*., 2012; Omid *et al*., 2015). The difference from the present study compared to the previous research might be associated with differences in age group, dietary habits among the children, environmental conditions and the data collection method. Most of the studies used a duplicate method or 3-day diary food record to obtain data whereas, in the present study, an FFQ was used to get dietary information. Miziara *et al*. (2009) used an FFQ in collecting dietary data, but the TDDFI was half of the value found in the present study. The high TDDFI might be due to the amount of food consumed and the halo effect as earlier discussed, since the drinking water fluoride concentration in the present study was low (0.04 mg/l) compared to the concentration experienced by the Brazilian children (0.6-0.8 mg/l).

When the TDDFI was adjusted by age in the Brazilian children (Miziara *et al*., 2009) and the present study, the TDDFI in the present study (0.060 mg/kgbw/d) was still twice the value (0.028 mg/kgbw/d) reported by Miziara *et al*. (2009). The difference could also be associated with differences in the dietary habits of the children in the present study and those

of the Brazilian children. Among the Brazilian children, the estimated fluoride contribution from the dietary components showed the highest contribution from water (0.011 mg/kgbw/d) whereas, in Nigeria, beverage consumption contributed more to the TDDFI. Also, the Brazilian study included very young children who eat less. No studies have reported the TDDFI of children in highly fluoride exposed population as obtained in this present study. The weight-adjusted TDDFI in the low fluoride area of the present study was also high compared to 0.019 mg/kgbw/d reported among Japanese children (Kimura *et al*., 2001), 0.039-0.046 mg/kgbw/d among Brazilian children (Lima and Cury, 2003) and 0.032-0.04 mg/kgbw/d for Columbian children (Franco *et al*., 2005; Franco *et al*., 2009). In studies conducted in the UK, the lowest TDDFI of 0.006 mg/kgbw/d was recorded in children up to 4 years old living in fluoridated area and uses toothpaste (Zohoori *et al*., 2013) and the highest (0.103 mg/kgbw/d) (Zohoori *et al*., 2014) was recorded in children less than 1-year-old living in a fluoridated area (0.97 mg F/l) (see Table 8.29). A study on infants in the USA aged 0.3-0.5 years also revealed fluoride intakes within the range of those recorded in the present low fluoride water area (0.07-0.16 mg/kgbw/d) (Wiatrowski *et al*., 1975). Interestingly, the results obtained in the present study among children for both low and high fluoride area is similar to those reported in fluoridated and non-fluoridated areas of the USA by Kramer at al. (1974). The non-fluoridated area in the USA has TDDFI range 0.78-1.03 mg/d whereas fluoridated area 1.73-3.44 mg/d (Kramer *et al*., 1974). Kramer *et al*. (1974) reported dietary fluoride intake was between 3 to 4 mg/d in the Chicago area, similar to the intake found in the high fluoride water area of the present study (3.738 mg/d). The high fluoride intake was associated with the dietary fluoride content and ingestion of artificial fluoridated drinking water, however, in the present study, the drinking water was naturally fluoridated.

The TDDFI of adults in the present study was significantly high in the high fluoride water areas (7.515 mg/d) compared to the low fluoride water area (1.904 mg/d). When the TDDFI was adjusted by weight, the was also significant difference between the low- (0.029 mg/kgbw/d) and high fluoride water area (0.120 mg/kgbw/d). The value from the low fluoride area was similar to intakes recorded for 31-45 and >50 year old adults in Chile (0.026-0.027 mg/kgbw/d), where the fluoride concentration in drinking water was 0.6 mg/l (Villa *et al*., 2004). No studies have reported fluoride intake from food in adults exposed to water that has a high F content.

8.17.7 Fluoride intake from toothpaste

The average weight of (1450 µg F/g) toothpaste dispensed by the children living in the low (0.266 g) and high (0.203 g) fluoride water areas was not statistically significantly different. Thus, the children in the present study used fluoridated toothpaste in line with the UK guidelines which recommend children aged 3-6 years old to take a pea-sized amount (approximately 0.25 g) of toothpaste containing 1350-1500 µg F/g (DoH/BASCD, 2009). Although, in the present study, all the children in the LFA brushed their teeth but majority (97%) of the children brushed once per day. The children might, therefore, be receiving less than the recommended fluoride intake from toothpaste. For example, the average amount of toothpaste dispensed by 4-6 years old UK children (Zohoori *et al*., 2012) is three times (0.67 g) the amount used by children in this study. Other studies also showed higher amounts of toothpaste dispensed by children compared to the present study: in Brazil among 1-3 year olds (0.49 g) and 4-7 year olds (0.43 g) (Pessan *et al*., 2003; De Almeida *et al*., 2007), 4 years old (0.45 g) children in Canada (Naccache *et al*., 1992) and 30 months old (0.36 g) English children (Bentley *et al*., 1999). This difference could be associated with the fact that in the majority of cases in the present study parents placed toothpaste on the toothbrush of their child (79% in the low fluoride water area and 100% in the high fluoride area). This is probably because they considered the cost of purchasing another tube when the toothpaste was exhausted. Socio-economic factors affect the rural populations in most developing countries. The level of dental awareness by parents might also be a factor considering that Brazil is also a developing country due to its low GDP.

Fluoride ingestion from toothpaste among children in the low- (0.228 mg/d) and high (0.191 mg/d) fluoride areas was not statistically significantly different in the present study. On a body weight basis, average fluoride intake from toothpaste ingestion in the present study was 0.014 mg/kgbw/d in both fluoride areas. Fluoride ingestion from toothpaste is low compared to previous studies with children: 4-6 year olds (0.029 mg/kgbw/d), 30 months old (0.06 mg/kgbw/d), 6-7 year olds (0.022 mg/kgbw/d), 1-3 year olds (0.03 mg/kgbw/d) and ≤4 years old children (0.037-0.055 mg/kgbw/d) in England (Bentley *et al*., 1999; Zohoori *et al*., 2006; Maguire *et al*., 2007; Zohoori *et al*., 2012; Zohoori *et al*., 2013), as well as 1.3-6 years old Colombian children (0.04 mg/kgbw/d) (Franco *et al*., 2009) and 2-6 years old Brazilian children (0.036 mg/kgbw/d) (Miziara *et al*., 2009) but was in the range of 0.01-0.04 mg/kgbw/d reported among 1.5-3.5 years old children in seven European countries (Cochran *et al*., 2004). The reason for the low ingestion from toothpaste might be

associated with the lower amount of toothpaste dispensed (0.203-0.266 g) on the toothbrush which was earlier described. Also, the fact that over 70% of children in the present study used toothpaste with a fluoride concentration above 1450 µg/g but brushed once per day. Most of the previous studies were conducted where there are strict guidelines, and strategies for their implementation are in place. Interestingly, the estimate of fluoride ingestion reported by Formon *et al*. (2000) for 4-5 year olds in USA, who brushed once per day with 0.55 g toothpaste containing 1000 µg F/g, was similar (0.009-0.013 mg/kgbw/d) to that recorded in the present study. However, in the present study, children dispensed less toothpaste, 0.20-0.27 g, but which contained more fluoride, 1450 µg/g.

There is currently no study on adult toothpaste ingestion. In the present study, the amount of (≥1450 µg F/g) toothpaste dispensed by adults who brushed once per day (about 90%) was 0.368 g and 0.468 g in the low- and high fluoride water area, respectively. Fluoride ingestion from toothpaste was 0.430 mg/d (0.006 mg/kgbw/d) and 0.452 mg/d (0.007 mg/kgbw/d) in the low- and high fluoride areas. Public Health England (2017) recommended that adults should brush their teeth at least twice a day with fluoride toothpaste containing 1350-1500 ppm.

In the present study, fluoride ingestion in children were twice the ingestion by adults. This result was in line with the report by MRC (2002), that ingestion by young children is greater than by adults because of their propensity to swallow fluoridated toothpastes rather than to spit it out (Osuji *et al*., 1988; Naccache *et al*., 1992).

8.17.8 Total daily fluoride intake (TDFI)

TDFI among children in the present study was statistically significantly different between the low- (1.214 mg/d) and high (3.896 mg/d) fluoride water areas. The value of TDFI reported in the low fluoride area is similar to the values among 1.3-6 years old children (1.308 mg/d) living in Columbia (Franco *et al*., 2009) and among 6-7 years old children (0.945-1.707 mg/d) in England (Zohoori *et al*., 2012). On adjustment for body weight, the total daily fluoride intake in all the studies was the same (0.08 mg/kgbw/d). It is essential to note that in the present study the highest contribution to the TDFI was from diet whereas, among the Columbian and English children, the highest contribution was from toothpaste ingestion. Earlier study conducted by Ophaug *et al*. (1985) concluded from some studies that a TDFI of 0.05-0.07 mg/kgbw/d is optimum for caries prevention in children. However, based on the effect of fluoride on dental fluorosis, EFSA (2005) set a Tolerable Upper Level

(UL) of 0.1 mg/kgbw/d for fluoride in children up to eight years. In the present study, despite the low concentration of fluoride in drinking water in the low fluoride water area (0.04 mg/l), 28.1% (Figure 8.1) and 34.4% of the children, respectively, are receiving an optimum and supra-optimum amount of fluoride. Surprisingly, 12.5% of the children are at risk of developing dental fluorosis due to their exposure to fluoride above the UL (>0.1 mg/kgbw/d) whereas 3.3% are at risk of developing dental caries.

The TDFI (1.214 mg/d) of children living in the low fluoride area in the present study is close to the value (1.02 mg/d) among 3-5 years old Chilean children (Villa *et al*., 2000) and among 2-6 years old Brazilian children (1.092 mg/d) where the fluoride intake was also estimated by FFQ (Miziara *et al*., 2009) and among 6-7 years old children (1.043 mg/d) in England who ingested milk containing 0.9 mg. Other studies reported lower intake as shown in Table 8.28 (Brunetti and Newbrun (1983); Ekstrand *et al*., 1984, 1994; Guha-Chowdhury *et al*., 1996; Zohoori and Rugg-Gunn, 2000; Haftenberger *et al*., 2001; Zohoori *et al*., 2006) compared to the present study.

The present study is the first report on fluoride intake among children in a population exposed to a high level of fluoride in the water supply. The TDFI of children living in the present high fluoride area shows that they are at risk of developing fluorosis. Eighty percent of the children received fluoride above the UL of >0.1 mg/kgbw/d, and evidence of this was seen in all the children who participated in the study.

Adult TDFIs in the present study were statistically significantly different between the low- (2.321 mg/d) and high (7.889 mg/d) fluoride water areas. The corresponding values on a body weight basis were 0.036 and 0.125 mg/kgbw/d, respectively. The value obtained in the low fluoride area of the present study is high compared to that obtained in Santiago, Chile (1.82 mg/d) among female volunteers whose reported fluoride intake from water alone was 1.612 mg/d (Villa *et al*., 2008). However, the value is within the range reported by Villa *et al*. (2004) among 19-73 years old adults in Chile of both genders (1.77-2.48 mg/d for three age sub-groups) but more closely related to the age group 19-30 years old adults (2.48 mg/d) (Villa *et al*., 2004). The fluoride contribution from drinks (excluding water) (3.065 mg/d) is high compared to the other dietary components (water and solids) in the high fluoride area. This high intake could be associated with the high consumption (5460 ml/d) of a locally-prepared alcoholic drink which has a fluoride concentration of 5.176 mg F/l. It is not surprising to find that the percentage contribution to the TDDFI from food was 72% in the

low fluoride water area whereas, in the high fluoride water area, drinks (including water) contributed 66% to the TDDFI which resulted in the high TDFI value. The adult TDFI reported in the high fluoride area of the present study is within the range (5.40-13.79 mg/d) of earlier studies conducted in the US where adult males received fluoride supplementation, as shown in Table 8.28 (Spencer *et al*., 1970; Mehashwari *et al*., 1981). In the present study, 84% of the adults living in the low fluoride area were exposed to suboptimum fluoride intake (<0.05 mg/kgbw/d) whereas, in the high fluoride area, 50 % of the adults were exposed to fluoride above the UL (≥0.1 mg/kgbw/d). Virtually all adults living in the high fluoride water area showed evidence of dental fluorosis.

Table 8.29 Previous studies of fluoride intake (different dietary and toothpaste assessment methods were used to generate the information)

Report	Age (year)	Source of fluoride intake (mg/day)			Total fluoride intake	
		Diet	TP ingestion	Supplement NaF (mg)	mg/day	mg/kgbw/day
Spencer *et al* (1970)	30-53[a]			21	13.79	
Wiatrowski *et al* (1975)	≤1 0.3-0.5	0.32 1.23				
Maheshwari *et al*. (1981)	20-45[a]			5 10	5.40 10.38	
Brunetti and Newbrun (1983)	3-4				0.33	
Ekstrand *et al*. (1984)					0.011 0.927	
Schamschula *et al*. (1988)	3-4	0.22 0.72				
Ekstrand *et al*. (1994)					0.190	
Guha-Chowdhury *et al*. (1996)	3-4	0.15	0.34		0.49	0.027
Villa *et al*. (2000)	3-5				1.02	
Zohoori and Rugg-Gunn 2000	4	0.390	0.104		0.426	0.030
Kimura *et al*. (2001)	1-6	0.28				
Haftenberger *et al*. (2001)	3-6	0.203	0.274		0.931	0.053

Report	Age (year)	Source of fluoride intake (mg/day)			Total fluoride intake	
		Diet	TP ingestion	Supplement NaF (mg)	mg/day	mg/kgbw/day
Lima and Cury 2003	1.7-2.5					
Tomori *et al*. (2004)	0.3-0.7					
Franco *et al*. (2005)	4-5				1.55	
Franco *et al*. (2005)	1.8-2.9				1.38	0.11
Zohoori *et al*. (2006a)	1-3	0.26	0.45		0.71	0.05
Zohoori *et al*. (2006b)	6-7	0.591 0.349 0.188				
Nohno *et al*. (2006)*	2-5	18				0.025 0.012
	6-8	20				0.025 0.014
Maguire *et al*. (2007)	6-7	0.188 0.349 0.565	0.549 0.534 0.478		0.736 0.883 1.043	0.031 0.038 0.047
Villa *et al*. (2008)	20-40[b]				1.82	
Franco *et al*. (2009)	1.3-6	0.602	0.706		1.308	0.08
Miziara *et al* (2009)	2-6	0.478	0.614		1.092	0.064
Zohoori *et al*. (2012a)	4-6		0.61			

Report	Age (year)	Source of fluoride intake (mg/day)			Total fluoride intake	
		Diet	TP ingestion	Supplement NaF (mg)	mg/day	mg/kgbw/day
Zohoori *et al*. (2012b)	6-7	0.341 0.578	0.606 1.130		0.945 1.707	0.038 0.076
Zohoori *et al*. (2013a)	≤4					0.048[c], 0.015[d] 0.061[c], 0.011[d]
Zohoori *et al* (2013b)	≤1					0.107 0.024
Zohoori *et al*. (2014)	≤1					0.107[g] 0.024[h]
Present study	4-5 ≥20	0.985[i], 3.378[j] 1.904[i], 7.515[j]	0.228[i], 0.191[j] 0.430[i], 0.452j		1.214[i], 3.896[j] 2.321[i], 7.889[j]	0.075[i], 0.277[j] 0.036[i], 0.125[j]

[a]male volunteers, [b] female volunteers, [c] fluoride toothpaste user, [d] fluoride toothpaste non-user, *toothpaste intake was not determined, [e] hot season, [f] cold season, [g] fluoridated area (mean 0.97 mgF/l), [h] non-fluoridated area (mean 0.19 mgF/l), [i]low fluoride area (0.04 mg/l), [j]high fluoride area (3.05 mg/l)

The present study is the first conducted in Nigeria where total daily fluoride intake has been investigated as well as intake from the two primary dietary sources. None of the volunteers reported intake of fluoride supplements or used fluoride varnishes. The present study has been able to provide confirmation of earlier reports of prevalence of dental fluorosis (51%) among 12-15 years old children exposed to fluoride ≤0.4 mg/l in drinking water in central Nigeria (Plateau and Bauchi state) (El-Nadeef and Honkala, 1998), and among 40-50 years old adults in the Panyan area, Jos (Guskit, 2010) and in Jos east LGA (Jaryum, 2005), Plateau state exposed to <0.5 mg/l fluoride in drinking water. Akso and Zoakah (2008) also reported 12.1% dental fluorosis prevalence among 12-15 year olds children in central Plateau, Plateau state Nigeria exposed to 0.68 mg F/l in drinking water. The dental fluorosis

prevalence in the low fluoride water area despite the low fluoride concentration in drinking water might be associated with fluoride intake from food and the high consumption of tea which also contributed immensely to the dietary fluoride intake in the low fluoride water area. The reason could be that most foods consumed in this area are not locally produced: rice and tea bags, which contributed most to the TDFI, were mainly externally sourced.

It was also interesting to discover that about 3.3% and another 3.3% of children and 13.3% and 26.7% of adults were exposed to optimum and suboptimum fluoride intake respectively in the high fluoride area, despite the high fluoride exposure from the diet (see Figures 8.1 and 8.2). A study conducted by Wongdem *et al*. (2007) showed that some children, who were born and raised in the high fluoride area where the study was conducted, did not show evidence of dental fluorosis, which they associated with a genetic predisposition. It can also be inferred from this study that they were not exposed to an excess amount of fluoride capable of development of dental fluorosis even though they have lived in the high fluoride area since birth as shown in a number of participants of this study (see Figure 8.32). This might be associated with the dietary habit of these children and adults living in the high fluoride area. Further studies might be necessary to investigate the relationship between gene and fluorosis.

Figure 8.32 Adult with no evidence of dental fluorosis born and raised in the high fluoride area (3.05 mg F/l in drinking water)

8.17.9 Contemporary fluoride biomarkers

8.17.9.1 Urinary fluoride excretion

Urine volume: The mean urinary volume corrected for 24 hours was 445 and 631 ml/d in the low- and high fluoride water areas respectively. Similar volumes were reported in

children: 400-1217 ml/d reported among 3-6 years old German children (Haftenberger at al., 2001); 406 ml/d among preschool Chilean children, (Villa *et al*., 2000), 610 ml/d among 3 and 4 years old Swiz children (Marthaler *et al*., 2000); 633 ml/d in 4-6 years old southern Texas children (Baez *et al*., 2000); 439.5 ml/d among 1.3-6 years old Venezuelan children, 675.3 ml/d among 3-5 years old Australian children (Crosby and Shepherd, 1957); 465 ml/d among 4 years old Iranian children (Zohoori and Rugg-Gunn *et al*., 2000); 504 and 461ml/d among 4 year old Sri Lankan and English children, respectively (Rugg-Gunn *et al*., 1993). The mean corrected urine volume in the present study among children living in the low fluoride area was statistically significantly different (<0.05) compared to that of those living in the high fluoride area. Likewise, variation in the urine volume among children of similar age in all the previous study identified above might be associated with differences in fluid intake (Crosby and Sheppard, 1957).

Surprisingly, there was a significant difference ($p<0.05$) in the corrected volume of urine between adults and children in the high fluoride area and low fluoride water area. Adults in the high fluoride area urinated more (1441 ml/d) than those in the low fluoride area (1048 ml/d), similar to what was found among the children, but the volume for adults was twice the amount for the children. Also, considering the amount of fluid consumed, the adults in the high fluoride area had more to drink than those in the low fluoride area but the difference was not significant. The high fluid consumption might be associated with the consumption of alcohol in the high fluoride area (Tables 8.11a and 8.14a show that 13 adults and nine children drank alcohol in the high fluoride area whereas none did in the low fluoride area). Eggleton (1942) revealed that 1g of alcohol drunk increases the urine by 10 ml following the fact that alcohol is diuretic. Therefore, high alcohol consumption in the high fluoride water area might have led to the observed increase in volume of urine excretion.

Daily urinary fluoride excretion (DUFE): In the present study, there was statistically significant difference in the urinary fluoride concentration between the low- (0.175 mg/l) and high (5.696 µg/ml) fluoride water area. The mean DUFE of children in the present study was 0.071 and 3.110 mg/d in the low- and high fluoride water area respectively. It is evident that higher fluoride intake from the diet resulted in higher fluoride excretion in urine. The value of DUFE in the low fluoride area of the present study is low compared to previously published studies where values range from 0.188 to 0.750 mg/d among 3-7 year olds (see Table 2.2). The lowest DUFE was recorded among 3 years old children exposed to a drinking water fluoride concentration of 0.12 mg/l and fluoridated salt (60-90 mg/kg)

(Acevedo *et al*., 2007), while the highest was reported among 4-6 years old children consuming drinking water containing 1.0-1.3 mg F/l in school and 0.1-3.2 mg F/l at home (Baez *et al*., 2000). Ekstrand *et al*. (1984) reported a lower DUFE value (0.030 mg/l) among 0.19-0.54 year old children with 1 mg F/l in their drinking water, compared to the present study, although the age group of the children was below the age group of participants in the present study. The reason for the differences observed between the present study and literature could be due to the low fluoride concentration (0.04 mg/l) of the drinking water in the low fluoride area of the present study compared to all the studies reported in Table 2.2. The DUFE in the high fluoride area of the present study (3.110 mg/d) is similar to that reported among 8-9 years old (3.100 mg/d) Mexican children (Grijalva-Haro *et al*., 2001). Interestingly, the Mexican children were exposed to drinking water containing 2.77 mg/l fluoride, similar to that reported in the high fluoride area of the present study (3.05 mg/l).

The mean adult DUFE in the present study was 0.355 and 8.658 mg/d in low- and high fluoride water areas, respectively. There is a large difference in the DUFE between the low fluoride area and the high fluoride area consistent with the corresponding large difference in TDFI values (2.321 mg/d and 7.889 mg/d respectively). As reported for children, the adult DUFE in the low fluoride area is low compared to the high fluoride area value. Few studies have reported DUFE values in adults. Buzalaf *et al*. (2006) reported among adults aged 20-35 years DUFEs of 0.952 and 1.642 mg/d in control and when exposed to a "fluoride-containing diet, a fluoridated dentifrice and fluoridated solution (1.8 mg fluoride)" respectively. Also, 1.24 mg/d DUFE was reported among 20-40 years old adults with diet intake of 1.82 mg/d (Villa *et al*., 2008). These lower DUFE values might be associated with the age-related differences between these published studies and the present work. A recent study conducted among 10-50 year olds in the Ethiopian Rift Valley drinking naturally contaminated groundwater with varying fluoride concentrations (0.6-15 mg/l) reported urinary fluoride excretion of 5.7 mg/12-h urine (estimated at 24-h DUFE was 11.4 mg/d). However, more than 55% of the participants had a DUFE similar to the 8.658 mg/d reported in the high fluoride area of the present study. There might be some bias to the estimated DUFE in Ethiopian studies as 12-h urine samples (overnight, 6pm to 6am) were collected (Rango *et al*., 2017). The concentration of fluoride in urine varies at different times of day and particularly when the urine is collected about 3 hours after a meal (WHO, 2014). Spencer *et al*. (1970) reported a similar DUFE value (7.52 mg/d) among 30-53 years old males in the USA who had a daily fluoride intake of 13.79 mg/d (including NaF

supplementation) and a value of 6.76 mg/d among 20-45 years old males who had a daily fluoride intake of 10.38 (including 10 mg fluoride supplementation). Urine pH should be considered when comparing studies of urinary fluoride excretion and factors that affect urinary pH including the composition of the diet, drugs, altitude and certain disease (Whitford, 1990, 1996; Buzalaf *et al*., 2011) as they affect the amount of fluoride that is excreted in the urine. An increase in urinary pH, increase excretion of fluoride in urine and subsequently lead to reduction in fluoride retention (Whitford *et al*., 1976).

When the DUFEs in adults and children were adjusted by weight, the mean DUFE of children (0.004 mg/kgbw/d) living in the low fluoride area was similar to that of adults (0.005 mg/kgbw/d), despite differences in their fluoride intake. In contrast, in the high fluoride area, the mean DUFE of children (0.210 mg/kgbw/d) was higher than that of the adults (0.140 mg/kgbw/d). The present study shows that there is age-related difference in the proportion of ingested fluoride that is excreted in urine. For children with low fluoride intake, a negative balance can occur, when the proportions of fluoride that is excreted in urine exceed the intake (MRC, 2002). There is net immobilization of fluoride from calcified tissue when there is a decline in plasma fluoride concentration due to reduction in the level of fluoride intake (Whitford, 1999). Zohoori *et al*. (2013) reported similar DUFEs of 0.005 and 0.008 mg/kgbw/d among fluoride toothpaste users and non-users, respectively, in a non-fluoridated area of Brazil (Joao Pessoa, with <0.0003 mg F/l in drinking water) for children up to 4 years old.

Correlation between daily urinary fluoride excretion (DUFE) and exposure (TDFI): The risk of dental and skeletal fluorosis might be determined more easily if there is a valid prediction of fluoride intake from measurement of urinary fluoride excretion (Villa *et al*., 2010). The present study investigated the suitability of DUFE as a biomarker of TDFI in children and adults. The results (Figure 8.3) show a highly significant linear relationship between DUFE and TDFI for children and adults, however, their respective intercepts and slopes were different. Villa *et al*. (2010) in an examination of the relationship between DUFE and TDFI for adults and children from published and unpublished reports with measurements of TDFI and DUFE, also showed different slopes and intercepts respectively in adults (0.540 and 0.290) and young children (0.350 and 0.03). In the present study, the respective slope and intercept was 0.647 and -0.008 in children whereas in adults, 0.687 and 0.017. The differences in the intercept and slope in the present study and the collective

examination of previous data conducted by Villa *et al*. (2010) might be associated differences in age, diet type, number of sample replicates. There was negative intercept in children indicating that when total daily fluoride is zero, children excreted very low concentration of fluoride. In the present study, DUFE was also strongly positively correlated with the fluoride content of the home water supply, contrary to what was reported by Maguire *et al*. (2007) among 6-7 years old children living in optimally, sub-optimally and non-fluoridated areas of the northeast of England. The UFE correlation with drinking water might be because children in the present study did not attend school and they drank from only one source of water, compared to the English children who were exposed to another source of water most of the day in school. Also, the number of participants investigated in the study (Maguire *et al*., 2007) was very small, therefore the margin of error is wide in the study. However, the two studies showed a strong relationship between DUFE and TDFI, suggesting that daily urinary fluoride excretion is a useful biomarker of contemporary fluoride exposure. A significant, though weak, correlation between urinary fluoride excretion and fluoride intake was reported among 4-6 years old Brazilian children compared to the present study (Buzalaf *et al*., 2011). The weak correlation might be associated with incomplete 24-hour urine sample collection, since no validation of complete urine collection was done in that study and intake from diets was based on research published two years before urine sample collection. There might have been a change in the dietary habits of the children: consumption of different food which had not been reported earlier.

8.17.9.2 Plasma fluoride concentration

Few studies have reported the fluoride concentration of blood plasma in children (Whitford *et al*., 1999; Levy *et al*., 2004) and none from an endemic fluoride water area. The present study found a statistically significantly higher mean (115.53 ng/ml) in children living in high fluoride water area. The concentration of fluoride in plasma in the low fluoride area children in the present study is higher than the value reported among 5-10 years old Georgia children, USA (16.9 ng/ml) (Whitford *et al*., 1999) and among 2-6 years old Brazilian children (19.0 and 24.0 ng/ml) exposed to drinking water fluoride 0.6-0.8 and 0.1-0.2 mg/l respectively (Levy *et al*., 2004). The difference form the latter study might be associated with fluoride intake following from the fact that the total daily fluoride intake in the present study (1.214 mg/d in the low fluoride area) was at least twice the range of values reported by Levy *et al*. (2004) (0.088-0.551 mg/d).

In adults, there was statistically significant difference in the plasma fluoride concentration between the low- (30.75 ng/ml (1.618 µmol/l)) and high (126.80 ng/ml (6.674 µmol/l)) fluoride water area (Tables 8.20 and 8.21). The plasma fluoride concentration in the low fluoride area is close to the estimated value (35 mg/ml (1.84 µmol/l)) reported among 10-38 years old adults who consumed fluoridated water (9.60 mg/l) in Sweden (Ekstrand, 1978). Whitford *et al*. (2008) reported a plasma fluoride concentration of 137.2 ng/ml (7.22 µmol/l) among 24-32 years old adults that were given fluoride dose (2.73 mg) in Brazil (naturally fluoridated water is 0.85 mg F/l). Other studies (see Table 2.1, chapter 2) conducted among adults (19-38 years old) showed lower values (6.8-22 ng/ml (0.36-1.16 µmol/l)) compared to the present study. This might be associated with the total daily fluoride intake and dietary habits which could influence rates of absorption and excretion as well as genetics (Carvalho *et al*., 2009).

There was no significant difference between the plasma fluoride concentrations of adults and children in the low fluoride water area as well as in the high fluoride water area in the present study. This could be associated to the fact that children and adults were from the same community with similar dietary habits/pattern, genetic and environmental conditions.

Correlation between plasma fluoride concentration and fluoride exposure (TDFI): In the present study, a positive statistically significant correlation was found (Figure 8.7) between plasma fluoride concentration and total daily fluoride intake in both children and adults suggesting that plasma fluoride concentration in a useful biomarker of contemporary fluoride exposure. A stronger relationship was found in the present study between plasma fluoride concentration and the fluoride content of primary drinking water sources reflecting the importance of plasma as a biomarker of intake for both adults and children. However, to be considered a viable biomarker capable of predicting fluoride exposure, the overall intake should be considered. A study conducted among 2-6 years old children living in fluoridated and non-fluoridated areas of Brazil showed no significant relationship associated with non-fasting children included in the study (Levy *et al*., 2004). Also, Ekstrand *et al*. (1994) associated the failure to demonstrate an effect of fluoride intake on plasma fluoride concentration in their control studies with the relatively low level of fluoride intake. There is possibility that the number of volunteers enrolled in the study was not sufficient to allow the detection of any difference with low exposure to fluoride and considering the differences in their diets. In the present study, there was no age-related difference between adults than

children, although plasma fluoride concentration in adults was slightly higher numerically than children in both fluoride areas. Blood plasma fluoride concentration can be inferred as the gold standard method among contemporary markers and resting plasma fluoride concentration reflects the level of fluoride in bone, which contains more than 99% of the body burden of fluoride.

8.17.9.3 Saliva fluoride concentration

The whole saliva collected in the present study was unstimulated, which was difficult to achieve with some children and adults due to low salivary rate. Some of the participant took a longer time to drool into the sampling container, however, in the present study, the time participant taken by to drool sufficient sample into the container was not investigated. Samples were analysed by the direct method after centrifuging to remove food debris, soft tissue cells etc. Fluoride concentration in the saliva is beneficial for the topical effect of fluoride in the oral environment during the processes of demineralisation and remineralisation (Zero *et al*., 1992; Vogel *et al*., 1992). Mean whole saliva fluoride concentration in the present study among children was 8.393 ng/ml (0.405 µmol/l) and 304.23 ng/ml (16.012 µmol/l) in the low- and high fluoride water areas, respectively. The saliva fluoride concentration in the low fluoride area is similar to the value (9.3 ng/ml (0.49 µmol/l)) obtained among six subjects after cellular debris and mucus were removed by centrifugation of the samples as in the present study (Yao and Gron, 1970) but above the range 2.5-7.8 µmol/l reported by previous studies in adults and children (Jacobson *et al*., 1992; Boros *et al*., 1999; Whitford *et al*., 1999). The concentration of fluoride in saliva is influenced by the amount of fluoride the subject is receiving from drinking water whether fluoridated or non-fluoridated (Oliveby *et al*., 1990; Hedman *et al*., 2006). Other factors that affect whole saliva fluoride concentration are fluoride intake from different sources such as food, beverages and fluoride supplements (Whitford, 1996). A higher saliva fluoride concentration (13.5 ng/ml (0.71 µmol/l)) was also reported by Oliveby *et al*. (1989) among 5-10 years old children possibly because saliva was analysed as collected in the subjects without removal of any food debris.

In the present study, the saliva fluoride concentration was three times lower than plasma concentration in the low fluoride area whereas in the high fluoride area saliva fluoride was about three times higher than plasma. Studies have shown that the concentration of fluoride in saliva is higher in communities with a high level of fluoride in the water supply than in

those with a low level of fluoride in the water supply (Yao and Gron, 1970, Oliveby *et al*., 1990). However, in the present study, the increase in the level of fluoride even though samples were collected from fasting subjects could be associated to fluoride retention in oral environment. Zoro *et al*. (1988) showed a prolonged fluoride retention in whole saliva with night-time fluoride application compared to day-time fluoride application. Boros *et al*. (2001) reported that fluoride concentration in whole saliva increases due to Sodium reabsorption with concomitant increase in water reabsorption during resting condition on passing through the luminal region of salivary glands. This is interesting, since the participants in this study provided overnight fasting samples.

In the present study, a similar concentration of fluoride in whole saliva was found in adults living in the low fluoride area and their children. In the high fluoride area, whole saliva fluoride concentration was higher in the children than adults. Interestingly, a similar relationship observed between plasma and whole saliva in low and high fluoride area among children was also found among the adults. There are few studies of whole saliva fluoride concentration in adults. Fukushima *et al*. (2011) reported whole saliva fluoride concentration (18-200 ng/ml) among 30-40 year olds and 16-231 ng/ml among 50-60 years old Brazilians living in non- (0.09-0.15 mg F/l), artificial (0.66 mg F/l) and naturally fluoridated areas (0.72-1.68 mg F/l). These reported values are higher than that observed in the low fluoride area of the present study. The value (231 ng/ml) reported in Brejo dos Santos, a naturally fluoridated urban community with drinking water fluoride 0.72 mg/l (Fukushima *et al*. 2011) is similar to the mean whole saliva fluoride concentration found in the high fluoride area (239.3 ng/ml) in the present study. Obviously, these communities are exposed to other sources of fluoride, as reported in the present study.

The whole saliva fluoride concentrations were not affected by age but water fluoride concentration was the main factor influencing whole saliva fluoride levels similar to previous findings (Zero *et al*., 1992; Fukushima *et al*., 2011).

Correlation between whole saliva fluoride concentration and fluoride exposure (TDFI): In the present study, a weak correlation was found between fluoride in whole saliva and total daily fluoride intake in both adults and children, although the correlation in adults was weaker. Similarly, with drinking water fluoride, there was a moderate relationship with saliva fluoride in children and a weak correlation in adults. Studies have revealed that diets and fluoridated dentifrice have great influence on the level of fluoride in the oral cavities

which would reflect in the concentration of fluoride in whole saliva (Rolla and Ekstrand, 1996). Martinez-Mier and Soto-Rojas (2010), also showed that the amount and frequency of fluid consumption influenced the concentration of fluoride in saliva. Fukushima *et al*. (2011) also did not find a dose-response relationship between fluoride concentration in water and whole saliva fluoride concentration. Also, Boros *et al*. (2001) showed a 2-fold increase with intake of 500 ml milk containing 2.5 mg fluoride but a 4-fold increase was observed with the intake of 200 ml milk containing 1 mg fluoride. They associated the relationship between intake and fluoride concentration of whole saliva to local effects of fluoride e.g. soft tissue retention of fluoride, dissolution of calcium fluoride, crevicular fluid, oral bacteria etc. (Boros *et al*., 2001). Therefore, weak relationship found in the present study show that whole saliva is not a good biomarker of systemic exposure to fluoride. However, some studies have shown whole saliva as a good biomarker of exposure but in these studies, fluoride concentration in whole saliva was measured after a recent exposure. Further evidence suggests a large pool of fluoride within the mouth associated to oral soft tissue and dental plaque, not easily removed by solution containing fluoride (Whitford *et al*., 1999). Therefore, whole saliva might be a better indicator of recent topical exposure (Oliveby *et al*., 1989; Duckworth *et al*., 1991; Zero *et al*., 1992; Cury *et al*., 2005; Whitford and Bawden, 2007; Duckworth *et al*., 2009; Szekely *et al*., 2010). Evidence from previous study has suggested parotid ductal saliva as a more appropriate biomarker of fluoride exposure than whole saliva (Whitford *et al*., 1999) but due to the practicalities of obtaining the sample, this was not investigated in the present study. Fluoride in saliva, particularly parotid saliva, has been reported in previous studies as a useful biomarker of fluoride intake due to its relationship with plasma fluoride concentration (Taves, 1968; Schamschula *et al*., 1985; Richard *et al*., 1992) but studies have not been able to establish a relationship between whole saliva and plasma (Whitford *et al*., 1999). Fluoride concentration in whole saliva was reported to be influenced by fluoride in the oral environment due to its ratio (10:1) with plasma fluoride (Oliveby *et al*., 1989) and a higher concentration compared to ductal saliva fluoride (Whitford *et al*. 1999; Martinez-Mier and Soto-Rojas, 2010; Fukushima *et al*., 2011).

8.17.10 Recent fluoride biomarkers

Biomarkers including nails (fingernail and toenail) and hair can be used in assessing sub-chronic exposure to fluoride. The level of fluoride in these biomarkers reflects intake during

their formation. The fluoride ingested in the body is absorbed into the bloodstream, is retained in calcified tissues and eliminated in the urine. Therefore, fluoride in nails and hair reflects past blood fluoride concentration and the overall body burden of fluoride (Rugg-Gunn *et al*. 2011), 3 months for nail (Whitford, 1996) but hair needs to be investigated.

8.17.10.1 Nail fluoride concentration

Mean fingernail fluoride concentration in children was 3.237 and 12.583 µg/g in the low- and high fluoride water areas, respectively. There was a significant difference in the level of fluoride in fingernails between the two areas, which suggests that intake is the primary determinant of the amount of fluoride in nail samples. The value obtained in the low fluoride area is within the ranges reported in previous studies: 2.22-3.53 µg/g for 1-3 years old children with a mean TDFI of 0.130 mg/d (de Almeida *et al*., 2007), 2.3-3.6 µg/g for 2-6 years old Brazilian children exposed to fluoride in drinking water ranging from 0.1 to 0.8 mg/l (Levy *et al*., 2004), 2.89-3.43 µg/g for 5-6 years old Brazilian children who received toothpaste containing fluoride between 550 and 1100 µg/g and pH range 4.5-7 (Buzalaf *et al*., 2009), 2.76-3.91 µg/g for 1-3 years old children with TDFIs between 0.036 and 0.043 mg/kgbw/d (Lima-Arsati *et al*., 2010), 1.93-3.38 µg/g for 4-6 years old Brazilian children (Amaral *et al*., 2014) recorded a very high value of 6.09 µg/g when the participants consumed fluoridated salt, and 2.44-3.26 µg/g for 1.5-2.5 years old children in Brazil) with total dietary fluoride intake of 0.025-0.040 mg/d (Amaral *et al*., 2014).

The present study is the first where fingernail- and toenail fluoride concentrations have been investigated in an endemic fluoride water area with children. The value of fluoride in nails (fingernail and toenail) among children living in the high fluoride area might serve to reflect children at risk to dental fluorosis in a population.

In the present study, mean toenail fluoride concentration among children was 3.957 and 11.381 µg/g in the low- and high fluoride water areas, respectively. Fingernail fluoride has been suggested to indicate chronic exposure to fluoride. Fingernails may be more prone to external contamination compared to toenails (Buzalaf *et al*., 2009) except on occasions where children walk bare footed particularly in the rural settings of some developing countries. Open toe shoes are very common in Africa, Asia etc. Therefore, there are chances of external contamination for individuals who wear open toe shoes as the nails might be exposed to dust, particularly when the soil has a high fluoride content. According to

Whitford (1999), toenails have been shown to have a lower fluoride content than fingernails and this was attributed to a greater supply of blood in fingernails. The toenail fluoride concentrations recorded in both areas in the present study are higher than what has previously been reported by Levy *et al*. (2004) among 2-6 year old children (1.6-2.8 µg/g) consuming water with 0.1-0.8 mg/l fluoride in Brazil. Buzalaf *et al*. (2009) found significant differences in toenail fluoride concentration among 5-6 year olds randomly allocated into groups depending on their consumption of dentifrice (experimental liquid dentifrice with different concentration and pH or commercial toothpaste) and also in another study among 4-6 years old children, Buzalaf *et al*. (2011) associated differences in the nail fluoride concentration in each group exposed to different fluoride source (artificial and natural fluoridated water, fluoridated salt; fluoridated milk and no systemic exposure) with their identified source of exposure. Buzalaf *et al*. (2011) reported highest toenail concentration in participants consuming fluoridated salt (180-200 mg F/kg), therefore, in the present study, high fluoride in toenail may be associated with the exposure to fluoride from various sources. A more recent study in Brazil among children 1.5-2.5 years old given dentifrice with different fluoride concentration and active ingredient reported toenail fluoride concentration (2.19-2.64 µg/g) half of what was reported in the present study (Amaral *et al*., 2014). The high nail fluoride content (fingernail and toenail) observed in the low fluoride area, as well as in the high fluoride area, show that nail fluoride concentration reflects fluoride intake associated with the area. Another factor that can influence nail fluoride concentration is the geographical area; at higher temperatures, people drink more water: which consequently leads to higher fluoride intake (Fukushima *et al*., 2009).

The concentration of fluoride in the fingernails, as well as in the toenails, of adults was similar to that of children, as earlier reported by Fukushima *et al*. (2009), who found that there were no age-related differences in the fluoride concentration in fingernails and toenails. The mean concentration of fluoride in the adults' fingernails was 2.805 and 9.415 µg/g in the low- and high fluoride water areas, respectively, whereas the corresponding toenail fluoride concentrations were 2.386 and 10.207 µg/g. The difference between the high- and low fluoride area values was significant for both toenails and fingernails. The first study on adults exposed to fluoride from drinking water (0.1-1.0 mg/l) showed high fluoride content in toenails (4.2-6.4 µg/g) (Spate *et al*., 1994), higher than the value reported in the low fluoride water area here. However, the above toenail values of Spate *et al*. (1994) are lower than the value recorded in the high fluoride area of the present study. The

difference might be due to the differences in intake from the major sources of fluoride (diet and toothpaste), forms of fluoride and its bioavailability. A recent study conducted in India in an endemic fluoride area (4.1-5.6 mg/l drinking water) showed very high fluoride concentrations in toenail samples (82.38-103.92 µg/g) (Sankhala *et al*., 2014). The reason for these exceptionally high values compared to the present study is not known since other sources of fluoride ingestion were not reported. In contrast, the mean fingernail fluoride concentration among adults in the low fluoride area of the present study (2.805 µg/g) is similar to the value reported among 25-50 years old stay-at-home mothers (2.75 µg/g) living in a volcanic area (Azores) of Portugal (Linhares *et al*., 2016). Therefore, participant in the low fluoride area were exposed to high level of fluoride similar to that reported in Portugal, however, the major source of exposure to fluoride in the present study was from food whereas in Portugal water (1.71 mg F/l). A recent study (Rango *et al*., 2016) conducted among 10-50 year olds living in an endemic fluoride area (Ethiopian Rift valley) reported fingernail fluoride (5.3 µg/g) less than the concentration found in the present study, and this might be due to the wide variation in the intake of fluoride between the two studies.

The present study showed that the fluoride content of toenails was higher than that of fingernails in adults living in both the high- and low fluoride areas, which is contrary to what was earlier reported by Whitford (1999), that increased blood flow in fingernails could lead to a higher fluoride content than in toenails. However, a recent study conducted among young adults in North Carolina, USA showed that toenails may reflect a long exposure time frame due to their relatively slow growth rate (3.47 vs 1.62 mm/month for fingernails and toenails respectively) (Yaemsiri *et al*. 2010). The difference in growth rate could be the reason why in the present study toenail fluoride concentration was higher than the corresponding fluoride concentration in fingernails, since more fluoride could be retained during the growth period. But this was not same among children living in the high fluoride area where toenail fluoride content was 1 µg/g less than fingernail fluoride content. The reason for the small difference in toenail and fingernail in the high fluoride area could be due to the length of time in which these children clip their nails since the children living in the high fluoride area do not go to school and none will be checking their nails. Another extrinsic factor could be due to most of the children playing with dust, when any resultant effect would be felt more in the high fluoride area. Nail fluoride concentration is a reflection of average level of intake and plasma concentration over protracted period and depends on frequency of nail clippings (Pessan and Buzalaf, 2011). Rodrigues *et al*. (2004) also

estimated slower growth rate in thumbnails among children aged 2-3 years old. In the present study, participants were not given a definite time to keep their nails before cutting, so the length of clipped nails varied between participants. Toenails might, therefore, be used for monitoring more prolonged exposure to fluoride in adults compared to fingernails. The differences between the present study and other studies might be associated with the length of nails before collection used in these separate studies since fluoride is incorporated into nails via the growth end (Whitford, 2005). More research needs to be conducted to investigate the average level of fluoride intake as well as the protracted period of plasma fluoride concentration reflected in the toenails. It was interesting to find age-related differences in fingernail and not in toenail. This might be attributed to the fact that fingernails grow much faster than they do in adults, about 50% faster (Tosti and Pitaccini, 2000; Berker and Baran, 2007). This could be the case where there is no condition of poor nutrition which retards the growth of nail since keratin the builds up the nail is a protein.

The present research shows that either fingernails or toenails could be used as a long-term biomarker of fluoride, but further research needs to be conducted to know the amount of fluoride present in either fingernail or toenail at a given protracted period.

Correlation between nail (fingernail and toenail) fluoride concentration and fluoride exposure (TDFI): In the present study, a moderate relationship was found between fingernail fluoride concentration and TDFI in adults (r=0.506) and in children (r=0.448) and similar associations were found for toenail fluoride concentration. However, stronger relationships were found between fingernail fluoride concentration and drinking water fluoride in both children and adults; likewise, for toenail fluoride. This finding suggests that fingernails and toenails are useful indicators of fluoride intake among groups of individuals (adults or children). Stronger correlations between fluoride concentration of nail and TDFI were established in this study in adults and children compared to what was reported among 4-6 years old children in Brazil (r=0.367 and 0.360 for fingernails and toenails respectively) (Buzalaf *et al*., 2011). The food data used by Buzalaf *et al*. (2011) were from earlier studies, so there might have been changes in the diet due to age for the participants whose current nail fluoride concentrations were investigated. Levy *et al*. (2004) showed a moderate correlation (r=0.57) between the pooled nail fluoride concentrations (fingernail and toenail) and estimated amount of ingested fluoride collected by a duplicate method among 2-6 year olds in fluoridated and non-fluoridated areas of Brazil. However, this author did not consider

the fact that fingernail and toenails will reflect different periods of exposure due to the differences in their growth rate. Levy *et al*. (2004) also found a positive statistically significant correlation (r=0.41; P=0.02) between fingernail and toenail fluoride concentration. In the present study, a much stronger statistically significant correlation was found between fingernail and toenail in adults (r=0.760; P<0.001) and similar relationship was found with children (r=0.611; P<0.001). We might conclude that fingernails and toenails may be used as biomarkers of sub-chronic fluoride exposure since in the present study all the primary dietary fluoride sources were considered. However, toenail and fingernail fluoride concentration represent different time frame of exposure. Therefore, both should not be pooled together during investigation of systemic exposure over a time-frame.

8.17.10.2 Hair fluoride concentration

Few studies have indicated that hair may be regarded as a biomarker of fluoride exposure (Buzalova, 1971; Schamschula *et al*., 1985, 1988; Czarnowski and Krechnaik, 1990; Kono *et al*., 1990, 1993; Mandinic *et al*., 2010). The utilisation of hair as a biomarker has been limited due to identified methodological issues: analytical methods; acceptability by subjects since sampling is done close to the scalp; the problem of external contamination; and interpretation of the results or other factors not identified by potential users; racial bias. In the present study, the mean fluoride concentration in the hair of children was 0.743 and 1.831 µg/g in the low- and high fluoride water areas, respectively, whilst the corresponding values for adults were 1.368 and 5.690 µg/g. To the researcher knowledge, the present study is the first study conducted among children aged 4-5 years where hair is investigated as a biomarker for monitoring exposure to fluoride. An earlier study (Schamschula *et al*., 1985) among 14 years old Hungarian children, found 0.18-0.40 µg/g in hair, lower than that reported in the low fluoride area of the present study. The lower hair fluoride concentration reported by Schamschula *et al*. (1985) might be due to their collection of the hair samples from the coronal region. The abundant supply of blood in the head is in the occipital region. Therefore, more fluoride circulating in the blood (Bassindale, 2012) is incorporated in the hair in occipital region compared to the coronal area (Harkey and Handerson, 1988). Also, a constant number (about 85%) of hair follicles are in the growing phase in the coronal region and hair in this site are less influenced by age and sex (Harkey and Handerson, 1988). In addition, there might be greater number of apocrine glands, a totally different *telogen/anagen* ratio (Kintz, 2004). A more recent study, conducted among 12 years old children from different Serbian municipalities (0.10-11 mg/l well water and 0.07-0.15 mg/l

tap water) (Mandinic *et al*., 2010), reported fluoride concentrations in the range 19.3-32.5 µg/g in their hair. In a different study among 7-15 year olds in Serbia exposed to water fluoride 0.11-4.14 mg/l, hair fluoride concentrations were 1.07-19.83 µg/g (Antonijevic *et al*., 2016). Therefore, interpretation of this study, as well as comparison with previous literature, should be done with care due to age-related differences in the hair fluoride concentration as shown in the present study between children and adults. The differences in fluoride content of hair between adults and children in the present study might be due to the differences in the utilisation of fluoride in bone by children compared to adults. Hair grow in cycles, alternating between period of growth (*anagen* phase and catagen phase (a short transition period where cell division stops and the follicle that is actively producing hair begins to deganerates)) and quiescence (telogen phase) a transition phase when the hair shaft stops growing completely and hair growth begins to shut down (Kintz, 2004). Therefore, the rate of hair growth and length of quiescent period vary with age, gender, race, disease states, nutritional deficiencies (Kintz, 2004). For children aged 4-8 years within the age group of children in the current study, head hair grows at the rate of 0.22-0.52 mm/day or 0.6-1.4 cm/month (Saitoh *et al*., 1969).

Other differences in fluoride intake was reported in the present study as well as other studies (Kokot and Drzewiecki, 2000; Mandinic *et al*., 2010). It is evident from the present study that water fluoride concentration affects fluoride concentration in hair as suggested by earlier studies (Czarnowski *et al*., 1999; Kokot and Drzewiecki, 2000; Wang *et al*., 2009; Parimi *et al*., 2013). However, none of the previous studies indicated other sources of fluoride exposure as reported in the present study. Gender differences were not investigated in the present study, but Parimi *et al*. (2013) showed no significant difference between gender among 30 people living in endemic- and low fluoride areas in India, but in their study, gender were not equally distributed (63% male and 37% female) for such conclusion to be made. Similarly, males in India keep their hair like the women do whereas in African populations, males regularly cut their hair while the females keep their hair. A report showed that it takes 10-14 days for drugs and alcohol to be deposited in the hair root and emerge past the scalp line. However, Kintz (2004) reported that surveillance of drugs in hair can take weeks to months depending on the length of the hair shaft but that most drugs can be tested for between 2-4 days. There might be the need for further investigation of the relationship between fluoride concentration in hair and the length of hair growth. It is necessary to know the accurate growth rate of hair, this would be particularly useful to define

precisely the time of fluoride exposure and to properly classify what type of biomarker hair would be.

The mean hair fluoride concentration of adults living in the high fluoride water area of the present study (5.69 µg/g) was similar to the values found among phosphate fertilizer workers (5.4 µg/g) (Czarnowski and Krechniak, 1990) and among 59 persons aged 17-87 years whose hair fluoride was determined after their death (5.2 µg/g) in the Gdansk region of Poland (Hac *et al*., 1997), although the cause of death and health status before death was not reported.

Correlation between hair fluoride concentration and fluoride exposure (TDFI): This is the first known report on the relationship between estimated total daily fluoride intake and fluoride concentration in hair. Evidence from the present study shows that hair may be useful for evaluating exposure to fluoride. A strong correlation (r=0.605) was found between hair fluoride concentration and total daily fluoride intake in adults whereas a weak correlation (r=0.306) was found in children. Corresponding relationships were also observed between hair fluoride concentration and drinking water fluoride concentration in adults (r=0.689) and children (r=0.420). As earlier mentioned, the weaker correlation in children might be associated with the utilization of fluoride by children for developing bones as well as other extrinstic factors (contamination from dust). None of the earlier studies on the use of hair as a biomarker of fluoride exposure reported a relationship with total daily fluoride intake. However, from the present study we found a consistent pattern of correlation between hair fluoride and total fluoride intake as well between hair fluoride and drinking water fluoride, so the present discovery is significant for future hair fluoride evaluation. Mandinic *et al*. (2010) also showed varying correlation between hair fluoride concentration and well water fluoride concentration as well as hair fluoride concentration and tap water fluoride concentration respectively in non-fluorotic region (Valjevo (r=0.89 and 0.91); Veliko Gradiste (r=0.97 and 0.53), Kacarevo (r=0.99 and 0.51)) and fluorotic (fluorosis endemic) region (r=0.54 and 0.34) in Serbia. There were strong correlations with well water fluoride concentration in all the non-fluorotic regions but a moderate correlation between hair fluoride concentration and tap water fluoride concentration in two non-fluorotic area. In the fluorotic region, a moderate correlation was found between hair fluoride concentration and well water fluoride concentration and a weak correlation with tap water fluoride concentration. This might be associated with other contributions to the fluoride intake

particularly in the fluorotic region since the same water will be used in preparing food thereby contributing to the overall intake. A recent study among 7-15 years old school children in Belgrade, Serbia, revealed a very strong correlation (r=0.94) between hair fluoride concentration and fluoride in drinking water (Antonijevic *et al*. 2015). However, a lower correlation (r=0.240) with drinking water fluoride was reported among 300 adults living in Gdansk region, Poland (Czarnowski *et al*., 1999), which might be associated with the use of a direct method for analysis of hair fluoride in the study, external contamination and whether different source of water is used for cooking. Elements in the hair, including fluoride, are bound to the sulphuric amino acid cysteine in the hair and this bound fluoride cannot be detected by ion selective electrode unless it is separated from the matrix using some pre-treatment methods. Particularly useful and more acceptable is the overnight diffusion method with acid-HMDS (Whitford *et al*., 1999). Due to the relationship found between hair fluoride concentration and TDFI in the present study, hair can be suggested as a biomarker for recent, sub-chronic or chronic fluoride exposure particularly in adults due to the stronger correlation.

8.17.11 RELATIONSHIP BETWEEN BIOLOGICAL MARKERS

Fluoride not deposited in the calcified tissues, following fluoride exposure, is excreted in the urine through the kidneys and it has been recommended by the World Health Organization as a contemporary biomarker of fluoride exposure (WHO, 2014). Plasma fluoride concentration when the subject is fasting may be a useful biomarker for estimating chronic fluoride intake or potential bone fluoride concentration (Ericsson *et al*., 1973). As discussed in the earlier sections for evaluating short-term fluoride exposures, fluoride concentration in bodily fluids including saliva, plasma and urine have proven to be very viable due to the relationships established with the fluoride intake. However, some factors have been reported to affect each of these biomarkers, particularly urine and saliva fluoride concentration. In the present study, in children, there was a strong correlation between TDFI and DUFE as well as with plasma fluoride concentration, a moderate relationship between TDFI and fingernail as well as with toenail fluoride concentration, and a weak correlation between TDFI and saliva as well as with hair fluoride concentration. In adults, there was a strong correlation between TDFI and DUFE, plasma and hair fluoride concentration, a moderate correlation between TDFI and fingernail as well as with toenail fluoride concentration.

Plasma: In the present study, in children, a further investigation showed a relationship between the recent biomarkers which reflect a different period of systematic fluoride exposure, between recent and the contemporary markers, and between contemporary biomarkers. Since the rates of blood flow to the different tissues govern the rates of fluoride distribution to these tissues (Ekstrand, 1996). The present study found in children a strong correlation between plasma and DUFE, a moderate correlation between plasma and fingernail as well as with toenail fluoride concentration, and a weak correlation between plasma and hair as well as saliva fluoride concentration. In adults, the study found a strong correlation between plasma and DUFE, hair and fingernail, a moderate correlation between plasma and toenail, and a weak correlation between plasma and saliva fluoride concentration. Therefore, we might conclude that plasma fluoride concentration in a fasting subject is the best biomarker among contemporary markers. However, when considering fluoridation programs, urinary fluoride excretion could be utilised due to the practical difficulties in obtaining blood from children and associated ethical issues.

In the present study, in children, we found a weak correlation between whole saliva fluoride concentration and DUFE, plasma and fingernail fluoride concentration, a moderate correlation between saliva fluoride concentration and toenail, and no relationship between whole saliva and hair fluoride concentration. Similarly, in adults, the study found a weak correlation between saliva and plasma as well as DUFE, and no relationship between saliva fluoride concentration and fingernail, toenail and hair fluoride concentration. Therefore, whole saliva is not a good biomarker for both children and adults but due to the weak correlations observed with the TDFI and other biomarkers. Some authors have shown poor correlations between whole saliva fluoride and plasma fluoride but found fluoride in ductal saliva to be more useful for comparison with plasma fluoride concentration (Whitford *et al*., 1999; Rugg-Gunn *et al*., 2011) since it was better correlated than the whole saliva. However, it should be noted that whole saliva F level is normally greater than ductal saliva F (and therefore should be easier to measure) but values are confounded by contributions from various oral sources of F as well as systemic F. Conversely, Boros *et al*. (2001) showed that fluoride ingested with milk failed to locally influence the whole saliva fluoride level during his studies where subjects refrained from fluoride-rich foods and drinks prior to and throughout the experiment and used herbal dentifrice without fluoride.

Hair: In children, the study found a moderate correlation between hair fluoride concentration and fingernail as well as with toenail, a weak correlation between hair and

DUFE as well as with plasma, and no relationship between hair and saliva. In adults, the study found a strong correlation between hair and DUFE, plasma and fingernail fluoride concentration, a moderate correlation between hair fluoride concentration and toenail, but no relationship between hair fluoride concentration and saliva fluoride concentration. Kono *et al*. (1990) also showed a strong correlation (r=0.650) among 142 hydrofluoric acid exposed workers between hair fluoride and post-shift urine fluoride.

Nail: In children, the study found a strong correlation between fingernail and toenail fluoride concentration, a moderate correlation between fingernail and plasma as well as with hair fluoride concentration, and a weak correlation between fingernail and DUFE as well as with saliva fluoride concentration. In adults, the study found a strong correlation between fingernail and plasma as well as with toenail fluoride concentration, a moderate correlation between fingernail and DUFE, and no relationship between fingernail and saliva fluoride concentration. With toenail fluoride concentration, the study found a moderate correlation between toenail and DUFE, saliva, plasma and hair fluoride concentration in children. Also, the study found a moderate correlation between toenail and DUFE, plasma and hair fluoride concentration in adults, but no relationship with saliva fluoride concentration.

Nail fluoride might be a useful biomarker compared to hair fluoride particularly in epidemiological studies when both adults and children are investigated and for fluoridation programmes when the risk of dental caries is considered. Some studies have investigated the relationship between nail and urinary fluoride excretion, which is the most used biomarker by WHO for monitoring fluoride exposure, and nail fluoride. Shankhala *et al*. (2014) showed a strong correlation (r=0.692) between toenail fluoride and DUFE among 40 years old adults in India. A weak association was reported between fingernail fluoride and DUFE (r=0.237) as well as toenail fluoride and DUFE (r=0.221) among 4-6 years old children receiving fluoride from different sources in Brazil (Czarnowski *et al*., 1999). From the result of the present study between nail (toenail and fingernail) fluoride concentration and DUFE in adults and children, we might conclude that nails are a useful biomarker among the recent biomarkers of fluoride exposure. Of interest, fingernail fluoride correlated strongly with toenail fluoride in both adults (r=0.611) and children (r=0.760) in the present study. A strong correlation was also observed by Buzalaf *et al*. (2011) (r=0.730) among 4-6 years old Brazilian children exposed to different sources of systemic fluoride. In contrast, Levy *et al*. (2004) found a weak correlation between fingernail fluoride and toenail fluoride among 2-6 years old children in Brazil (r=0.401), which might be associated with the differences in

toenail fluoride and fingernail fluoride content since they would reflect different periods of fluoride exposure due to their dissimilar growth rates.

Therefore, we could infer from the present study that nail fluoride might be a better biomarker of fluoride exposure among children and adults for the long term when different age groups are considered together. However, individually toenail and fingernail represent different exposure periods in children and adults. This needs to be further investigated to know the length of time toenail represent in children and adult (see recommendations in Chapter 10).

8.17.12 Influence of age and fluoride area on the various fluoride biomarkers

The present study was a two-way factorial design with two independent variables, age and fluoride area, and each biomarker as the dependent variable. We investigated the effect of age differences (adults and children) and fluoride water area (high fluoride area and low fluoride area) on saliva fluoride concentration, urinary fluoride excretion, plasma fluoride concentration, hair concentration, fingernail concentration and toenail concentration. We found that age group, whether adult or child, did not influence the fluoride concentration of some of the biomarkers including: saliva fluoride concentration, plasma fluoride concentration, and toenail fluoride concentration. However, fluoride area influenced the concentration of all the biomarkers. A study conducted among different age groups (3-7, 14-20, 30-40, 50-60 years) in 5 Brazilian communities showed that geographical area and water fluoride concentration exerted much influence on fingernail and toenail fluoride concentrations (Fukushima *et al*. 2009). We might, therefore, infer that the fluoride concentration of the supply water used for cooking and drinking in an area will influence fluoride exposure, which will in-turn alter the level of fluoride in these biomarkers of exposure. Although the utilisation of some of these biomarkers are not affected by age, careful consideration should be given when considering the use of biomarkers that are affected by age in epidemiological studies. Fukushima *et al*. (2009) found age-related difference in fingernail as well as toenail fluoride concentrations.

8.17.13 Limitation of study

8.17.13.1 Bias based on the food assessment tool

The researcher could not use the duplicate method which is the gold standard method for dietary assessment due to the number of participants involved in the current study. There

would have been a burden on parents (to collect the samples) and on the researcher (to analyse so many samples). The duplicate plate method is usually used for small-scale studies. Also, the cost of providing duplicate food samples from 120 participants would have been mostly borne by the researcher. Therefore, a validated food frequency questionnaire was used to collect the data. This meant that there were chances of overestimation or underestimation of the consumption from the diet, mainly from children who when at school might have consumed specific foods which might not have been reported by the parents. A few of the children who participated in the current study attended school. Similarly, the weight of food consumed was perceived by the parents. However, the researcher used plate measures collected from the homes of some of the participants and this was assumed as the general types of plates used in the study location. Also, due to the practicality of storing collected food before analysis, the researcher could not collect and prepare all the food identified in the FFQ. Therefore, the fluoride concentration of foods and drinks not commonly consumed were estimated from previous studies (Appendix 22) in the area, and from other studies in different locations (Cutrufelli *et al*., 2004; Zohoori and Maguire, 2015; Ibiyemi *et al*., 2016). This procedure assumed fluoride concentrations which might have some effect on the overall intake reported in the study. However, this was the only practical way to obtain the information.

8.17.13.2 Bias based on the estimation of toothpaste in adults

The assumed percentage of 41% for ingestion of toothpaste by adults used in the present study was from data obtained in children. This percentage was used due to the lack of data for adults and considering the recommendation "spit don't rinse" by a group of experts who formulated consensus statements regarding rinsing behaviour and the prevention of dental caries (Pitts et al. 2012). Therefore, there was overestimation of adult ingestion of toothpaste, since adults do not have the immature swallowing reflex of children. However, in the current study, the TDFI among adults was 0.035 and 0.127 mg/kgbw/d in the LFA and HFA respectively and the contribution of toothpaste to the TDFI in both low and high fluoride areas was 0.006 and 0.007 mg/kgbw/d respectively which is relatively small, representing 7% and 5% of TDFI in the LFA and HFA respectively. Hence, the difference in intake is not associated with toothpaste ingestion in the two fluoride areas.

8.17.14 Conclusions

On the basis of relationship with the total daily fluoride intake, the present study concluded that plasma is the most reliable biomarker of exposure to fluoride. Urinary fluoride excretion was also a good contemporary biomarker of exposure among children and adults similar to plasma but age should be considered when utilising UFE as a biomarker. Conversely, saliva is not a good biomarker of exposure to fluoride among children and adults. The present study also showed that nail both fingernail and toenails is good biomarker of sub-chronic/chronic exposure to fluoride, however, toenail represents a longer period of exposure to fluoride compared to fingernail and the concentration of fluoride in toenails is not affected by age. Similarly, hair might be regarded as a good biomarker of exposure to fluoride but further studies needs to be conducted to determine the period of exposure it reflects so as to effectively classify whether it is a recent, sub-chronic or chronic biomarker.

The present showed that the brushing habit of children was influenced by the level of parent's education and awareness of the importance of toothbrushing. Parents choice of toothpaste was not based on guidance from informed policy but the popularity of the brand and availability in the market informed their choices.

Contribution of fluoride in the present study was majorly from diet, interestingly most participants living in the low fluoride area received optimum amount of fluoride required to protect against dental caries therefore, most food consumed in the area might have been out sourced from areas where fluoride concentration in high. Most of the children living in the high fluoride area are at risk of developing dental fluorosis. It was not surprising to find that a few received below sub-optimum level in the high fluoride area as this evidence confirmed the findings of previous study which showed that not all people living in high fluoride area are at risk and the reasons for this is subject to further investigation.

CHAPTER 9: COMPARISON OF THE QUALITATIVE STUDIES IN THE UK AND NIGERIA

9.1 Introduction

This project aimed to assess and compare biomarkers of fluoride exposure (including plasma, urine, saliva, nails, hair) in adults and children. Although a specific biomarker can be a useful tool for assessment of fluoride exposure, it might not be acceptable to participants. Therefore, for a biomarker of fluoride exposure to be applicable in a large population, it should be readily collectable without objections from the donors. Also, there might be possible differences in people's choice of biomarker due to cultural differences. The perception of people on the use and collection of the different biological markers of exposure to fluoride in the UK and Nigeria has been presented in chapters 5 and 7, respectively. The present chapter compares the level of acceptability for the collection of biomarkers among these two populations (UK and Nigeria).

9.2 Aim

The aim of this chapter is to compare people's perception of biomarker collection in the UK and Nigeria.

9.3 Participation and perception of biomarker collection among individuals in the UK and Nigeria

In the present study, the findings from Chapter 5 (qualitative study in UK) and 7 (qualitative study in Nigeria) showed two main differences between these two countries: participation in research and acceptability.

9.3.1 Recruitment and participation

A successful ethics approval was obtained from both the UK (appendix 1 and 3) and Nigeria (appendix 2 and 4). In Nigeria, the ethics approval was obtained through a local health service-based Institutional Review Board (IRB) in the state capital where the research was conducted, whereas in the UK ethics approval was obtained through a university-based IRB. The procedure for obtaining approval in both countries was similar regarding consent, compensation, anonymity and withdrawal of consent. However, in the UK, parents were required to sign a separate consent for their young children, if the child was not able to, whereas in Nigeria, parents consented for their children on the same consent form. Also, in

Nigeria it was mandatory to inform the IRB of the completion of the project and a copy should be retained in the administrative office of the IRB.

In the UK, the recruitment was planned through primary schools in Middlesbrough and through Teesside and Newcastle Universities. The researcher contacted the head teachers of primary schools through emails and followed up with phone calls (See Chapter 4). The recruitment officer of Teesside University also engaged some schools for participation in the study. In the UK study, a £1 book voucher was offered to schools as an incentive for every returned response form (either positive or negative) and £1each for participants who completed the questionnaire was also approved for the research, but the incentive did not motivate the neither the school nor participants and subsequently did not yield any result. However, despite these attempts, the school participation rate was not improved. Several reasons were given by head teachers for non-participating in the research, including busy schedules, level of involvement in other research, etc. (See Chapter 5).

Staff of Newcastle and Teesside University was contacted through an email containing a link to an online survey designed by the researcher on Bristol Online Survey (BOS). Despite a successful recruitment of university staff for the adult online survey, there was a sharp fall in the number of parents who completed the survey on behalf of their children. A total of 120 parents were contacted in 3 primary schools located in Middlesbrough, where the head teacher accepted to take part, among which 4% (5) completed the questionnaire. Whereas, in the online survey, a total of 134 participants (109 adults plus 25 parents who completed on behalf of their children) completed the survey, representing a 61% response rate.

In Nigeria, an online survey was not conducted but participants were recruited through the selected communities (See Chapter 6). All participants in the community were contacted, and volunteers were recruited until the sample size for the study was complete. Participants completed the questionnaire after they consented to the study, prior to participating in the quantitative studies (see Chapter 8). Therefore, all participants (100%) completed the questionnaire in Nigeria.

In the present study, we were not successful recruiting through primary schools in the UK. However, successful recruitment from the Universities could be associated with their level of awareness about research. Therefore, awareness about the implications of research findings played a crucial role in the recruitment of participants in the UK, considering the respondents willingness to participate in the research. In Nigeria, the researcher was very successful in recruiting participants. This success might be associated with the fact that the researcher understood the setting and adopted the six-step strategy for community

engagement which has been used for research participation in developing countries (Diallo *et al*., 2005) before the participants' consent. The process includes: 1) a study of the community (the setting, culture, religion etc.), 2) an introductory meeting with the community leaders, 3) a formal meeting with the leaders, 4) a personal visit to the leaders, 5) meeting with traditional health practitioners, and 6) recognition that obtaining permission is a dynamic process. Generally, in Africa, for successful implementation of a research project, consultation and approval from community leaders, elders and tribal chiefs precedes individual consent within a community (Molyneux *et al*., 2004; Marshall and Lavery, 2007). In the present study, in one of the communities, the recruitment and the retention of study participants was almost affected by rumours, a misconception about the intended purpose of the study, but the researcher continuously conducted sensitisation meetings with members of the community with the support of the community leaders.

"*Why are they asking for six samples (blood, saliva, urine, hair, nail and water), they are 666?*"

In this study, the rumors could be associated with the belief system of the people living in the study location as well as in other parts of Nigeria and Africa. Before westernization, Africans had always believed in gods (iron, water, thunder etc.) and ancestors had been profoundly spiritual (Makgobi, 2014). Westernization brought Christianity, which was practiced by all people in the community where the study was conducted. The number of the Beast (666), according to the Christian doctrine revealed in the Bible Book of Revelation, chapter 13, is associated with an unknown Antichrist. It is therefore not surprising why they linked the research project to such a belief.

9.3.2 Acceptability

The study in the UK found a higher preference for providing nail samples and to some extent blood among the adult participants. They would instead prefer non-invasive samples like hair and nails for their child, but the collection of blood was not acceptable. Similarly, in Nigeria, adult participants found the collection of blood acceptable, although they had a higher preference for saliva. Surprisingly, despite the non-invasive nature of the collection of hair and nails, they were less preferred compared to blood. Parents in Nigeria were also indifferent about the samples their child could provide, and they believed biological samples provided by themselves could also be given by their child if it concerned their wellbeing. This is because they were not too particular about alternatives available for such treatment;

all that mattered to them was that care was given to their children no matter the cost. In this regard, most parents had no concerns with their children providing blood for monitoring of exposure to fluoride. In both countries, previous experience of providing biological samples (e.g. for routine medical tests) was a critical factor in acceptability of the biomarker. Collection of nails and saliva was found acceptable by participants in both countries.

In the UK, participants' acceptance of blood could be associated with their previous experience in providing such samples. In Nigeria, the high preference for blood, despite the invasiveness of the sample collection, could be associated with parents' experience in particularly with the provision of blood for tests of typhoid and other diseases prevalent in Nigeria. Malaria and typhoid fever are among the common infectious diseases that are endemic in Nigeria (Ngwu and Agbo, 2003; Enabulele and Nymike, 2016). Typhoid fever, which is spread by a faecal-oral route (WHO, 2003), commonly shows some symptoms including fever, headache, joint pain, nausea, vomiting, constipation, and diarrhoea (Parry *et al*., 1999; Gasem *et al*., 2002). The above diseases are usually diagnosed by blood culture, especially in the first week of infection (WHO, 2003). In Nigeria and other parts of Africa, the Widal agglutination test is the most common diagnostic tool employed in the diagnosis of typhoid fever because it is relatively cheap, easy to perform and requires minimal training and equipment (Enabulele and Nymike, 2016).

With regards to hair, the main drawback in the UK was the creation of a patch on the scalp due to the loss of hair after sample collection. However, in Nigeria the main concern that was raised was not associated with self-image but with the fear that the samples could be used for a ritual purpose (see Chapter 5). Cultural practices also played a key role, as participants in the UK were not willing to provide hair samples, even though it was easy to collect and in a non-invasive way. The belief was that keeping of hair enhances beauty, even among males, whereas in Nigeria men as well as some women cut their hair. Therefore, in Nigeria participants would be willing to provide hair in future if they were not suspicious of the real intention of the researcher or why they were offering the samples in the laboratory.

In both countries, similar concerns were raised with regards to the use of 24-hour urine for monitoring exposure to fluoride, including work-related issues and the size of the sample container. Participants in both countries believed it would be difficult to collect the sample on a working day and they might be forced to stay at home for sample collection. The size of the sample collection container also made the procedure more difficult, as it might not be

possible carrying large containers to the workplace due to the difficulty of sample storage. They also believed it would be difficult monitoring their children to collect the 24-hour urine samples. Another issue raised by the UK participants, which did not matter to those in Nigeria, concerned hygiene-related matters. In sub-Saharan Africa, 70% of people do not have access to a toilet, and according to the Japan International Cooperation agency (2013), the percentage of people without access to basic sanitation facilities was 37% in 2010 in Africa overall. Therefore, hygiene issues might not be a significant concern for the utilisation of 24-hour urine in Nigeria and Africa at large (See plate 9.1.

Figure 9.1 A typical rural Nigeria setting with no built toilets (https://ynaija.com/opinion-reversing-nigerias-deplorable-water-sanitation-hygiene-situation/)

The qualitative study found that participants' acceptance of biological markers of exposure to fluoride in the UK was governed mainly by their ease of use whereas, in Nigeria, approval was by perceived usefulness. Most people in developing countries have limited access to healthcare facilities. In Africa, approximately 1.6 million people died of malaria, tuberculosis and HIV-related illnesses in 2015 (UN Africa, 2016). In Nigeria, healthcare access is only 43.3% despite the various reforms to increase the provision of health (Onwujekwe *et al*., 2010). Healthcare in Nigeria is mostly provided by the private sector (70%) and 30% by the government (Omotuan *et al*., 2009), in a country where half of the population live below the poverty line, and on less than $1 a day. They cannot afford a high cost of health care (Steinberger *et al*., 2008). Therefore, it is evident that participants would

be indifferent to the choice of the samples used for monitoring a disease but would rather focus on the benefit associated with the collection of such biomarker samples. In the UK study, participants acknowledged the importance of the biomarkers for monitoring exposure to fluoride, especially related to the prevention of dental caries, yet they had very mixed views about their utilisation.

Lastly, cultural beliefs and practices play a crucial role in decision-making in most rural communities in Nigeria and the world at large. If the information is not adequately managed, it will affect the choice of biomarker or participants would not be willing to provide any sample. A study conducted among 12 Indian participants living in England, who did not donate blood, showed that "emotional charge", a situation where blood is much associated with family and acted as a symbol of kinship, opposed donated blood to be distributed to strangers (Joshi and Meakin, 2017).

9.3.3 Limitations

9.3.3.1 Type of questionnaire

The methodology adopted in the study both in the UK and Nigeria was a combination of web-based questionnaire and paper questionnaire depending on the practicality of data collection and study participants, as identified in Chapters 5 and 7. In the UK both web-based and a paper questionnaire was used for data collection whereas in Nigeria only a paper questionnaire was utilised. There might be chances that the participation in the survey might be influenced by the type of questionnaire completed by the participants (Drummond *et al*. 1995). High participation of the web-based questionnaire compared to the conventional paper questionnaire was found in the current study. Although the utilization of web-based questionnaires has some disadvantages including: uncertainity over the validity of data and sampling issues, concern around the design, implementation and evaluation of the online survey (Wright, 2005), this was managed in the current study by targeting recipents carefully and emphasising the value of their contribution to the study (Hunter, 2012). Studies have also shown that the results from web-based questionaires and paper questionnaires are comparable (Drummond *et al*. 1995; Stratton et al. 1998).

9.3.3.2 Deprivation status

The deprivation status in the UK was determined using IMD data found on the local council website and school as well as participants were classified based on the data. In this regard,

all children going to a school in a highly-deprived area would also be termed as highly deprived, similarly for the low-deprived area. However, since IMD would consider all possibilities, the limitation in the current study would be related to not classifying/grouping participants based on their socio-economic staus, not having equal number of pariticpants from different groups (children and adults), not randomly selected participants and more responses were from more educated people. However, in Nigeria, most of the participants had similar educational qualifications, socio-economic status, life style, religion, and occupation. In this regard, when comparing the two countries, we didn't match the number of participants from both countries based on their socio-economic, education levels, occupation, and lifestyle. This might therefore limit the comparison drawn between the two studies.

9.4 Conclusion

Interestingly, blood seems to be acceptable among adults in both the UK and Nigeria. However, when considering biomarkers for children, invasiveness of sampling technique, e.g. blood collection, might deter participants from enrolling their children into such studies. Therefore, non-invasive biomarkers of exposure to fluoride such as hair and nails should be considered. It is crucial to educate better and inform study participants about the need for sample collection. Overall, cultural beliefs and practices play crucial roles in peoples' perception on the choice of biological markers of exposure to fluoride in both the UK and Nigeria. Other factors that should be considered in future utilisation of the biomarkers, when conducting such a study in an African setting, are: level of awareness, peoples' experience and community engagement.

CHAPTER 10: CONCLUSIONS AND RECOMMENDATIONS

10.1 Introduction

This chapter presents the overall conclusions of the thesis, which are itemised separately under the qualitative study and quantitative study. The chapter is then concluded with further suggestions for future research.

10.2 Overall conclusions

10.2.1 Qualitative study

In the UK, the choice of biomarker of fluoride exposure was based on the ease of use whereas in Nigeria, perceived usefulness was the major determinant factor. In Nigeria blood was perceived the most acceptable biomarker of exposure to fluoride among adults and children whereas in the UK, nail was perceived the most acceptable biomarker of fluoride exposure for both age groups. However, adults in the UK would still be willing to provide blood samples due to previous experience in giving such sample. Overall, cultural beliefs and practices, as well as previous experience were important factors to consider in people's preferences of biomarkers of fluoride exposure.

10.2.2 Quantitative study

Majority of fluoride biomarkers have great potential for monitoring of exposures where the concentration of fluoride is low as well as in endemic areas in both children and adults.

Among contemporary and recent biomarkers, plasma is a more reliable biomarker since it reflects total daily fluoride intake in both children and adults even when exposure is very low. However, compared to the other types of biomarkers (including hair, nail, and urine), analytical precision of plasma was poor due to its low fluoride concentration. However, due to the invasive nature of collecting blood samples and ethical consideration as well as the pain particularly in children, the urine sample can be considered in place of plasma.

For long-term exposure, nail sample could be more reliable in both children and adults because they reflect plasma fluoride concentration over an extended period and are not affected by recent fluoride intake as well as specific physiological factors. Whole saliva is not a good biomarker of exposure to fluoride in adults and children due to the weak relationship with total daily fluoride intake both in children and adults.

Fluoride concentration of water influenced the fluoride concentration of all the biomarkers investigated including hair, fingernails, toenails, plasma, saliva and urine. Age also exerted influence on hair, fingernail and urine fluoride concentration.

10.3 Public health implications

Considering the benefit vis à vis side effects of high exposure to fluoride and the need to assess the benefit/risk ratio to maximise the benefit (prevention of dental caries) and minimise the risk (dental fluorosis/skeletal fluorosis), it becomes necessary to know the body burden of fluoride. Several other adverse effects, including osteosarcoma (Bucher et al., 1991; Bassin et al., 2006), developmental neurotoxicity (DHSS, 1991; NRC, 1993), immunological effects (Spittle, 1993) and reproductive toxicity (Erickson et al. 1976), have been suggested to be due to fluoride exposure, although these effects might only be observed at high exposure levels or at lower levels for an extended period (MRC, 2002), although, most of the evidence for these effects have not been substantiated (MRC, 2002). Therefore, WHO recommends that fluoride exposure should be monitored regularly as part of any fluoride supplementation (or prescription) programme in order to ensure that fluoride exposure in the population involved is at the appropriate level (WHO, 1994). Monitoring of fluoride exposure is particularly important considering the changing pattern of systemic fluoride exposure, to help researchers and policymakers to make a better-informed decision on the best ways to balance dental caries risk while preventing issues associated with high exposure to fluoride. It can also serve to assess the impact of water fluoridation on bone quality and other physiological conditions (O'Mullane et al., 2016) and therefore will be useful for public health administrators examining the population for health impacts of fluoridation.

Exposure assessment remains a critical step for studies on health effects, but current methods for monitoring fluoride exposure are based on community evaluations. Evidence on the health effects of fluoride has been centred almost entirely on fluoride ingested from drinking water without considerable information on other sources of fluoride exposure (MRC, 2002). Also, estimation of total exposure to fluoride following from the summation of intakes of food, drinks and water may be insufficient, overlooking other secondary sources of exposure considering that there is a relatively low therapeutic ratio between dental caries and fluorosis. Several diet assessment methods (Ophaung et al., 1980; Schamschula et al., 1988; Maguire et al., 2007; Miziara et al., 2009; Martinez-Mier and Soto-Rojas, 2010) have been

used in estimating fluoride exposure but each method is not without its challenges including cost, over- and under-reporting, misreporting or forgetfulness, coding errors or missing out a food type, high level of expertise (Miazira et al. 2009; Omid et al. 2015). Also, oral hygiene questionnaires have been used for estimating fluoride exposure from toothpaste, but there are also chances of over- or under- estimation. Although a valid and reproducible method has been used in measuring and analysing the amount of toothpaste dispensed during tooth brushing and expectorated saliva by some authors (Maguire et al., 2007; Franco et al., 2005), this method is usually time-consuming, costly and requires a high level of expertise.

According to the WHO, biomarkers play roles primarily for identifying efficient or deficient biologically available fluoride (WHO, 1994). Biological markers including tissues and fluids might be used with varying degrees of accuracy in estimating the body burden of fluoride at both community and individual level. Contemporary biomarkers including urine, saliva, plasma, milk, sweat are needed to estimate current exposure, while chronic fluoride exposure is assessed by historical biomarkers including bone, teeth, hair and nails.

10.3.1 Biomarkers of fluoride exposure in community evaluation

The findings in this study suggest several implications for policy and practice. Health authorities should not make a judgement based on the level of fluoride in drinking water alone or in the utilisation of toothpaste when deciding on fluoridation programmes. The current study highlighted the importance of biomarkers should any decision be made for fluoride supplementation or a fluoridation scheme. In this regard, health authorities could utilise the understanding of bioavailable fluoride associated with health effects in a community before general policy-making. These actions may help to monitor fluoride exposure effectively and identify the population at risk. Also, scientific questions, including the target populations, whether long-term or short-term monitoring and perception of the population, should guide the choice of biomarkers. Health authorities could work with researchers for general health messages particularly when making a recommendation for the introduction of fluoridation programmes vis à vis assessing the effectiveness of such a scheme. This study reveals biomarkers that would be useful for monitoring short-term and long-term fluoride exposure, and it would be useful for public health administrators to seek guidance from researchers before making decisions particularly in epidemiological studies which might involve thousands of subjects and considerable expense. Better planning and identification of relevant biomarkers as well as identification of confounding factors

associated with the use of such biomarkers are essential. Policymakers would be able to enact legislation regarding the utilisation of fluoride following from community evaluation of fluoride exposures using a biological marker.

10.3.2 Biomarkers of fluoride exposure for individual evaluation

Biomarkers could be useful for individual evaluations for health professionals when giving advice/recommendations at the individual level and identifying the population at risk. Samples of biomarkers assessed for fluoride exposure will inform medical/dental practices of the likely extent of occurrence (dental caries and fluorosis as well as other possible health effects) in individuals who might be affected as well as in the identification of any vulnerable subgroups. Also, such recommendations can be directed to the government, particularly when the population at risk is identified.

10.3.3 Relevance of public perception of biomarker

Perception of population groups varied with culture, experience, educational level and age. Health authorities and health practitioners should consider this when they are working with different population groups. A biomarker might be reliable but difficult to collect among some subgroups. For example, plasma collected from fasting subjects might be the best biomarker and acceptable among children and adults in a population like Nigeria but in a country like the UK, it might be difficult to use such a biomarker among children. In this regard, health authorities could consider urinary fluoride excretion which is also a useful biomarker. The understanding of community and individual perception of biological markers will foster participation in community-based programmes and epidemiological studies. It will also assist health care professionals during recommendation for individual assessment of exposure to fluoride.

10.4 Recommendations

This section presents some recommendations for Nigeria where the main study was undertaken

10.4.1 Dentistry

- Dentist in the low fluoride area should encourage parents to brush the teeth of their children twice per day and with the adequate amount (pea sized) of fluoridated toothpaste. On the contrary, dentist practicing in the high fluoride area should

discourage the use of fluoridated dental products that further increase the risk of dental fluorosis in children. This could be done during visit but since most people in the rural areas rarely visit the dentist, the practitioners could organise personally a visit to communities.

- They should continually advise government on the dangers of neglect particularly for children living in endemic fluoride area until steps are taken to either de-fluoridate their water or provide potable water with low fluoride content.

10.4.2 Ministry of Health

The Ministry of Health has the responsibility to facilitate the introduction in some area specific oral health initiatives that will promote good oral hygiene among parents/guardians and their children living in areas where drinking water fluoride concentration is low, with key objectives including:

- The need of national guidelines for toothpaste use among adults and children particularly in areas where the risk of dental caries is high.

- Facilitate provisions in government policy for strict enforcement of proper labelling of toothpaste products (guidelines for toothpaste use, amount of fluoride, active ingredient etc.) by companies.

- Facilitate the use of topical measures such as the introduction of fluoridated milk in schools located within affected areas as well as the distribution of free dental products in the rural areas where most parent's socio-economic level and education is low.

- Facilitate the teaching and awareness of dental hygiene in schools, mainly in primary schools, as well as local campaigns in rural communities on the benefits of good oral hygiene.

There is the need for the provision of de-fluoridated water in the high fluoride water areas, followed by an awareness of the dangers of consumption of naturally fluoridated water by children living in these areas. However, if the government would not be able to afford such a project due to the cost, an immediate measure should be taken such as the provision of affordable water with a low fluoride concentration for all children living in the area.

10.4.3 Toothpaste manufacturers

- The toothpaste manufacturers in Nigeria need to ensure that their products are correctly labelled according to international guidelines and when national guidelines become available, they should ensure strict compliance to the standards.
- Industry should support the government in promotional campaigns through television and radio commercials, community campaign programs, schools as well as social media to ensure parents understand the benefits of toothbrushing.

10.4.4 Recommendations for future studies

- A national survey on fluoride exposure is necessary to identify areas at risk of dental caries, or dental fluorosis in Nigeria since drinking/cooking water fluoride concentration alone is not sufficient in estimating the risk of these disease conditions. A national survey would further help to re-classify Nigeria based on dental caries and dental fluorosis risk prevalence.

- Further studies should be conducted by measuring dental caries and dental fluorosis level, if possible in the same participants recruited in the present study in Nigeria, to link the measured biomarker fluoride concentrations to their subsequent dental fluorosis or dental caries levels. The fluoride concentrations of biomarker or the high fluoride water area reported here will inform future studies of the upper limit of fluoride intake that might lead to the manifestation of dental fluorosis when children become adults.

- A study needs to be conducted to determine the differences in the protracted period of exposure to fluoride between fingernails and toenails considering the age-related discrepancies, particularly with fingernail fluoride. Also, a similar study should be conducted in the hair of both children and adults.

- There is the need for a repeat study on the influence of genes on the occurrence of dental fluorosis considering the challenges of the present study which affected the use of the field data.

REFERENCES

ABDULLAH, A., LIEW, S.M., HANAFI, N.S., NG, C.J., LAI, P.S., CHIA, Y.C. and LOO, C.K., 2016. What influences patients' acceptance of a blood pressure telemonitoring service in primary care? A qualitative study. *Patient Preference and Adherence,* **10**, pp. 99-106.

ABOU-ZEID, A., SILVERMAN, H., SHEHATA, M., SHAMS, M., ELSHABRAWY, M., HIFNAWY, T., RAHMAN, S.A., GALAL, B., SLEEM, H., MIKHAIL, N. and MOHARRAM, N., 2010. Collection, storage and use of blood samples for future research: views of Egyptian patients expressed in a cross-sectional survey. *Journal of Medical Ethics,* **36**(9), pp. 539-547.

ACEVEDO, A.M., FEBRES-CORDERO, C., FELDMAN, S., ARASME, M.A., PEDAUGA, D.F., GONZÁLEZ, H. and ROJAS-SÁNCHEZ, F., 2007. Urinary fluoride excretion in children aged 3 to 5 years exposed to fluoridated salt at 60 to 90 mgF/Kg in two Venezuelan cities. A pilot study. *Acta Odontol Latinoam,* **20**(1), pp. 9-16.

ADAIR, S.M. and WEI, S.H., 1978. Supplemental fluoride recommendations for infants based on dietary fluoride intake. *Caries Research,* **12**(2), pp. 76-82.

AFSSA (AGENCE FRANÇAISE DE SÉCURITÉ SANITAIRE DES ALIMENTS), 2003. *Rapport du Comité d'experts spécialisé "eaux" concernant la proposition de fixation d'une valeur limite du fluor dans les eaux minérales naturelles.* France: Afssa.

AKOSU, T.J. and ZOAKAH, A.I., 2008. Risk factors associated with dental fluorosis in Central Plateau State, Nigeria. *Community Dentistry and Oral Epidemiology,* **36**(2), pp. 144-148.

AKPATA, E., DANFILLO, I., OTOH, E. and MAFENI, J., 2009. Geographical mapping of fluoride levels in drinking water sources in Nigeria. *African Health Sciences,* **9**(4), pp. 227-233.

ALVAREZ, J.A., REZENDE, K., MAROCHO, S., ALVES, F., CELIBERTI, P. and CIAMPONI, A.L., 2009. Dental fluorosis: exposure, prevention and management. *Journal of Clinical and Experimental Dentistry,* **1**(1), pp. 14-18.

AMARAL, J.G., FREIRE, I.R., VALLE-NETO, E.F., CUNHA, R.F., MARTINHON, C.C. and DELBEM, A.C., 2014. Longitudinal evaluation of fluoride levels in nails of 18–30-month-old children that were using toothpastes with 500 and 1100 µg F/g. *Community Dentistry and Oral Epidemiology,* **42**(5), pp. 412-419.

ANGER, W.K., MOODY, L., BURG, J., BRIGHTWELL, W.S., TAYLOR, B.J., RUSSO, J.M., DICKERSON, N., SETZER, J.V., JOHNSON, B.L. AND HICKS, K., 1986. Neurobehavioral evaluation of soil and structural fumigators using methyl bromide and sulfuryl fluoride. *Neurotoxicology*, *7*(3), pp.137-156.

ANGMAR-MÅNSSON, B. and WHITFORD, G.M., 1990. Environmental and physiological factors affecting dental fluorosis. *Journal of Dental Research,* **69**, pp. 706-713.

ANTONIJEVIC, D., JESCHKE, A., COLOVIC, B., MILOVANOVIC, P., JEVREMOVIC, D., KISIC, D., VOM SCHEIDT, A., HAHN, M., AMLING, M. and JOKANOVIC, V., 2015. Addition of a fluoride-containing radiopacifier improves micromechanical and biological characteristics of modified calcium silicate cements. *Journal of Endodontics,* **41**(12), pp. 2050-2057.

AOBA, T. and FEJERSKOV, O., 2002. Dental fluorosis: chemistry and biology. *Critical Reviews in Oral Biology and Medicine,* **13**(2), pp. 155-170.

ARENDS, J. and CHRISTOFFERSEN, J., 1990. Nature and role of loosely bound fluoride in dental caries. *Journal of Dental Research,* **69(**2_suppl**)**, pp. 601-605.

ASDR, 2003. *Toxicological Profile for Fluorides, Hydrogen Fluoride, and Fluorine.* Atlanta, USA: Agency for Toxic Substance and Disease Registry.

ASHBURN, T.T., WILSON, S.K. and EISENSTEIN, B.I., 2000. Human tissue research in the genomic era of medicine: balancing individual and societal interests. *Archives of Internal Medicine,* **160**(22), pp. 3377-3384.

ATHANASOULES, T., 1978. Biological effects of large doses of fluorides on general health. *Odontostomatologike proodos,* **32**(2), pp. 90-101.

BAGLIO, M.L., BAXTER, S.D., GUINN, C.H., THOMPSON, W.O., SHAFFER, N.M. and FRYE, F.H., 2004. Assessment of interobserver reliability in nutrition studies that use direct observation of school meals. *Journal of the American Dietetic Association,* **104**(9), pp. 1385-1392.

BAILEY, F., CHILTON, J., DAHI, E., FEWTRELL, L. and MAGARA, Y., 2006. Fluoride in drinking water. World Health Organization. *Geneva, Switzerland*.

BAKER, R., 1972. Determination of fluoride in vegetation using the specific ion electrode. *Analytical Chemistry,* **44**(7), pp. 1326-1327.

BALAZOVA, G., 1971. Long term effect of fluoride emission upon children. *Fluoride,* **4**(2), pp. 85-88.

BANNISTER, L.H. and WILLIAMS, P.L., 1995. *Gray's Anatomy: the Anatomical Basis of Medicine and Surgery.* Churchill Livingstone. London, UK.

BARANOWSKI, T., DWORKIN, R., HENSKE, J.C., CLEARMAN, D.R., DUNN, J.K., NADER, P.R. and HOOKS, P.C., 1986. The accuracy of children's self-reports of diet: Family Health Project. *Journal of the American Dietetic Association,* **86**(10), pp. 1381-1385.

BARTNECK, C., SUZUKI, T., KANDA, T. and NOMURA, T., 2007. The influence of people's culture and prior experiences with Aibo on their attitude towards robots. *Ai & Society,* **21**(1-2), pp. 217-230.

BASIOTIS, P.P., WELSH, S.O., CRONIN, F.J., KELSAY, J.L. and MERTZ, W., 1987. Number of days of food intake records required to estimate individual and group nutrient intakes with defined confidence. *The Journal of Nutrition,* **117**(9), pp. 1638-1641.

BASSIN, E.B., WYPIJ, D., DAVIS, R.B. and MITTLEMAN, M.A., 2006. Age-specific fluoride exposure in drinking water and osteosarcoma (United States). *Cancer Causes & Control*, *17*(4), pp.421-428.

BECKER, C.B., DARIUS, E. and SCHAUMBERG, K., 2007. An analog study of patient preferences for exposure versus alternative treatments for posttraumatic stress disorder. *Behaviour Research and Therapy,* **45**(12), pp. 2861-2873.

BECKER, W. and BRUCE, A., 1981. Fluoride intake from food. *Var föda,* **33**(Suppl 3), pp. 198-261.

BELTRAN, E.D. and BURT, B.A., 1988. The pre-and posteruptive effects of fluoride in the caries decline. *Journal of Public Health Dentistry,* **48**(4), pp. 233-240.

BENFORD, D.J., HANLEY, A.B., BOTTRILL, K., OEHLSCHLAGER, S., BALLS, M., BRANCA, F., CASTEGNARO, J.J., DESCOTES, J., HEMMINIKI, K. and LINDSAY, D., 2000. Biomarkers as predictive tools in toxicity testing. *ATLA-NOTTINGHAM-,* **28**(1), pp. 119-132.

BENTLEY, E., ELLWOOD, R. and DAVIES, R., 1999. Oral hygiene: Fluoride ingestion from toothpaste by young children. *British Dental Journal,* **186**(9), pp. 460- 462.

BIAN, J.Y., WANG, W.H., WANG, W.J., RONG, W.S. and LO, E., 2003. Effect of fluoridated milk on caries in primary teeth: 21-month results. *Community Dentistry and Oral Epidemiology,* **31**(4), pp. 241-245.

BINKA, F.N., BAWAH, A.A., PHILLIPS, J.F., HODGSON, A., ADJUIK, M. and MACLEOD, B., 2007. Rapid achievement of the child survival millennium development goal: evidence from the Navrongo experiment in Northern Ghana. *Tropical Medicine and International Health,* **12**(5), pp. 578-593.

NUFFIELD COUNCIL ON BIOETHICS, 2011. *Human Bodies: Donation for medicine and research.* London: Nuffield Council report

BJÖRNSTRÖM, H., NAJI, S., SIMIC, D., SJÖSTRÖM, I. and TWETMAN, S., 2004. Fluoride levels in saliva and dental plaque after consumption of snacks prepared with fluoridated salt. *European Journal of Paediatric Dentistry,* **5**(1), pp. 41-45.

BOAHEN, O., OWUSU-AGYEI, S., FEBIR, L.G., TAWIAH, C., TAWIAH, T., AFARI, S. and NEWTON, S., 2013. Community perception and beliefs about blood draw for clinical research in Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **107**(4), pp. 261-265.

BOIVIN, G., CHAPUY, M., BAUD, C.A. and MEUNIER, P.J., 1988. Fluoride content in human iliac bone: results in controls, patients with fluorosis, and osteoporotic patients treated with fluoride. *Journal of Bone and Mineral Research,* **3**(5), pp. 497-502.

BONJOUR, J.P., CAVERZASIO, J. and RIZZOLI, R., 1993. Effect of fluoride on bone cells. *Research and Clinical Forums*. **15**, pp. 9-12.

BOROS, I., KESZLER, P. and BANOCZY, J., 2001. Fluoride concentrations of unstimulated whole and labial gland saliva in young adults after fluoride intake with milk. *Caries Research,* **35**(3), pp. 167-172.

BROFFITT, B., LEVY, S.M., WARREN, J.J. and HELLER, K.E., 2004. Seasonal variation in fluoride intake: the Iowa fluoride study. *Journal of public health dentistry,* **64**(4), pp. 198-204.

BRUNETTI, A., 1983. Fluoride balance of children 3 and 4 years old. *Caries Research,* **17**, p. 171.

BUCHER, J.R., HEJTMANCIK, M.R., TOFT, J.D., PERSING, R.L., EUSTIS, S.L. AND HASEMAN, J.K., 1991. Results and conclusions of the National Toxicology Program's rodent carcinogenicity studies with sodium fluoride. *International journal of cancer*, *48*(5), pp.733-737.

BURT, B.A., 1992. The changing patterns of systemic fluoride intake. *Journal of Dental Research,* **71**, pp. 1228-1237.

BURT, B.A. and EKLUND, S.A., 2005. *Dentistry, Dental Practice, and the Community.* Elsevier Health Sciences. UK.

BURTON, J.L. and WELLS, M., 2002. The Alder Hey affair. *Archives of Disease in Childhood,* **86**(1), pp. 4-7.

BUZALAF, M.A. AND WHITFORD, G.M., 2011. Fluoride metabolism. In, Buzalaf, M.A.R. ed., Fluoride and the Oral Environment. *Monographs in Oral Science,* **22**, pp. 20-36.

BUZALAF, M.A., CAROSELLI, E.E., CARDOSO DE OLIVEIRA, R., GRANJEIRO, J.M. and WHITFORD, G.M., 2004. Nail and bone surface as biomarker for acute fluoride exposure in rats. *Journal of Analytical Toxicology,* **28**, pp. 249-252.

BUZALAF, M.A. and LEVY, S.M., 2011. Fluoride intake of children: consideration of dental caries and dental fluorosis. In, Buzalaf, M.A.R. ed., Fluoride and the Oral Environment. *Monograph in Oral Science,* **22**, pp. 1-19.

BUZALAF, M.A., PESSAN, J.P., HONORIO, H.M. and TEN CATE, J.M., 2011. Mechanism of action of fluoride for caries control. In, Buzalaf, M.A.R. ed., Fluoride and the Oral Environment. *Monograph in Oral Science,* **22**, pp. 97-114.

BUZALAF, M.A.R., MASSARO, C.S., RODRIGUES, M.H.S., FUKUSHIMA, R., PESSAN, J.P., WHITFORD, G.M. and SAMPAIO, F.C., 2012. Validation of fingernail fluoride concentration as a predictor of risk for dental fluorosis. *Caries Research,* **46**, pp. 394-400.

BUZALAF, M.A.R., RODRIGUES, M.H.C., PESSAN, J.P., LEITE, A.L., ARANA, A., VILLENA, R.S., FORTE, F.D.S and SAMPAIO, F.C., 2011. Biomarkers of fluoride in children exposed to different sources of systematic fluoride. *Journal of Dental Research,* **90**(2), pp. 215-219.

BUZALAF, M., DE ALMEIDA, B., DA SILVA CARDOSO, V., OLYMPIO, K. and DE ALMEIDA FURLANI, T., 2004. Total and acid-soluble fluoride content of infant cereals, beverages and biscuits from Brazil. *Food Additives and Contaminants,* **21**(3), pp. 210-215.

BUZALAF, M.A.R., FUKUSHIMA, R., GRANJEIRO, J.M. and CURY, J.A., 2002. Correlation between plasma and nail fluoride concentrations in rats given different levels of fluoride in water. *Fluoride,* **35**(3), pp. 185-192.

BUZALAF, M.A., LEITE, A.L., CARVALHO, N.T., RODRIGUES, M.H., TAKAMORI, E.R., NICONIELO, D.B., LEVY, F.M. and CARDOSO, V.E., 2008. Bioavailability of fluoride administered as sodium fluoride or monofluorophosphate to humans. *Journal of Fluorine Chemistry,* **129**(8), pp. 691-694.

BUZALAF, M.A., PESSAN, J.P. and ALVES, K.M., 2006. Influence of growth rate and length on fluoride detection in human nails. *Caries Research,* **40**(3), pp. 231-238.

BUZALAF, M.A., VILHENA, F.V., IANO, F.G., GRIZZO, L., PESSAN, J.P., SAMPAIO, F.C. and OLIVEIRA, R.C., 2009. The effect of different fluoride concentrations and pH of dentifrices on plaque and nail fluoride levels in young children. *Caries Research,* **43**(2), pp. 142-146.

CALDERON, J., MACHADO, B., NAVARRO, M., CARRIZALES, L., ORTIZ, M.D. AND DIAZ-BARRIGA, F., 2000. Influence of fluoride exposure on reaction time and visuospatial organization in children. *Epidemiology*, 11(4), pp. S153-S153.

CALVERT, G.M., MUELLER, C.A., FAJEN, J.M., CHRISLIP, D.W., RUSSO, J., BRIGGLE, T., FLEMING, L.E., SURUDA, A.J. AND STEENLAND, K., 1998. Health effects associated with sulfuryl fluoride and methyl bromide exposure among structural fumigation workers. *American Journal of Public Health*, *88*(12), pp.1774-1780.

CAO, S., 1992. Study on preventive and control measures on coal-combustion type endemic fluorosis in the Three Gorges area in China. *Chinese Journal of Endemic Disease, II (Suppl.),* pp. 6-21.

CAO, S. and LI, Y., 1992. The evaluation of indoor air quality in areas of endemic fluorosis caused by coal combustion, *Proceedings of the XIX Conference of the International Society for Fluoride Research, Kyoto* 1992.

CAO, J., BAI, X., ZHAO, Y., LIU, J., ZHOU, D., FANG, S., JIA, M. and WU, J., 1996. The relationship of fluorosis and brick tea drinking in Chinese Tibetans. *Environmental Health Perspectives,* **104**(12), pp. 1340-1343.

CARDOSO, S.M., MOREIRA, P.I., AGOSTINHO, P., PEREIRA, C. and OLIVEIRA, C.R., 2005. Neurodegenerative pathways in Parkinson's disease: therapeutic strategies. *Current Drug Targets-CNS & Neurological Disorders,* **4**(4), pp. 405-419.

CARDOSO, V.E.S., WHITFORD, G.M. and BUZALAF, M.A.R., 2006. Relationship between daily fluoride intake from diet and the use of dentifrice and human plasma fluoride concentrations. *Archives of Oral Biology,* **51**, pp. 552-557.

CARDOSO, V.E., WHITFORD, G.M., AOYAMA, H. and BUZALAF, M.A., 2008. Daily variations in human plasma fluoride concentrations. *Journal of Fluorine Chemistry,* **129**(12), pp. 1193-1198.

CARVALHO, J.G.D., OLIVEIRA, R.C.D. and BUZALAF, M.A.R., 2006. Plasma as an indicator of bone fluoride levels in rats chronically exposed to fluoride. *Journal of Applied Oral Science,* **14**(4), pp. 238-241.

CARVALHO, J.G., LEITE, A.L., YAN, D., EVERETT, E.T., WHITFORD, G.M. and BUZALAF, M.A., 2009. Influence of genetic background on fluoride metabolism in mice. *Journal of Dental Research,* **88**(11), pp. 1054-1058.

CHACHRA, D., VIEIRA, A.P. and GRYNPAS, M.D., 2008. Fluoride and mineralized tissues. *Critical Reviews in Biomedical Engineering,* **36**(2-3), 183-223.

CHACHRA, D., TURNER, C.H., DUNIPACE, A.J. and GRYNPAS, M.D., 1999. The Effect of Fluoride Treatment on Bone Mineral in Rabbits. *Calcified Tissue International,* **64**(4), pp. 345-51.

CHATIO, S., BAIDEN, F., ACHANA, F.S., ODURO, A. and AKAZILI, J., 2016. Knowledge and perceptions about clinical trials and the use of biomedical samples: Findings from a qualitative study in rural Northern Ghana. *PloS one,* **11**(4), pp. e0152854.

CHEN CJ *ET AL*., 1988. A nationwide survey on drinking water quality and waterborne diseases in China. Beijing, Institute of Environmental Health and Monitoring, *Chinese Academy of Preventive Medicine*, pp. 95-99.

CHIBA, K., YOSHIDA, K., TANABE, K., OZAKI, M., HARAGUCHI, H., WINEFORDNER, J. and FUWA, K., 1982. Determination of ultratrace levels of fluorine in water and urine samples by a gas chromatographic/atmospheric pressure helium microwave induced plasma emission spectrometric system. *Analytical Chemistry,* **54**(4), pp. 761-764.

CHOUBISA, S., 2001. Endemic fluorosis in southern Rajasthan, India. *Fluoride,* **34**(1), pp. 61-70.

CHOUBISA, S., CHOUBISA, D., JOSHI, S. and CHOUBISA, L., 1997. Fluorosis in some tribal villages of Dungarpur district of Rajasthan, India. *Fluoride,* **30**(4), pp. 223-228.

CHOWDHURY, N.G., BROWN, R. and SHEPHERD, M., 1990. Fluoride intake of infants in New Zealand. *Journal of Dental Research,* **69**(12), pp. 1828-1833.

CLOVIS, J. and HARGREAVES, J.A., 1988. Fluoride intake from beverage consumption. *Community Dentistry and Oral Epidemiology,* **16**(1), pp. 11-15.

COLUCCI, A.C.A., PHILIPPI, S.T. and SLATER, B., 2004. Development of a food frequency questionnaire for children aged 2 to 5 years. *Revista Brasileira de Epidemiologia,* **7**(4), pp. 393-401.

CORREA RODRIGUES, M.H., DE MAGALHAES BASTOS, J.R. and RABELO BUZALAF, M.A., 2004. Fingernails and toenails as biomarkers of subchronic exposure to fluoride from dentifrice in 2- to 3-year-old children. *Caries Research,* **38**(2), pp. 109-114.

CRESSEY, P., GAW, S. and LOVE, J., 2010. Estimated dietary fluoride intake for New Zealanders. *Journal of Public Health Dentistry,* **70**(4), pp. 327-336.

CROSBY, N.D. and SHEPHERD, P.A., 1957. Studies on patterns of fluid intake, water balance and fluoride retention. *The Medical Journal of Australia,* **44**(9), pp. 305-311.

CURY, J., DEL FIOL, F., TENUTA, L. and ROSALEN, P., 2005. Low-fluoride dentifrice and gastrointestinal fluoride absorption after meals. *Journal of Dental Research,* **84**(12), pp. 1133-1137.

CUTRUFELLI, R., PEHRSSON, P., PATTERSON, K., WILGER, J., HAYTOWITZ, D. and HOLDEN, J., 2004. *USDA National Fluoride Database of Selected Beverages and Foods*. US.

CZARNOWSKI, W. AND KRECHNIAK, J., 1990. Fluoride in the urine, hair, and nails of phosphate fertilizer workers. *British Journal of Industrial Medicine,* **47**, pp. 349-351.

CZARNOWSKI, W. AND KRECHNIAK, J., 2002. Urinary fluoride of school children in Gdansk. *Fluoride,* **35**(4), pp. 239-243.

DABEKA RW, CONACHER HBS, SALMINEN J., NIXON GR, RIEDEL G., CROCKER R., 1992. Survey of bottled drinking water sold in Canada. Part 1. Lead, cadmium, arsenic, aluminum, and fluoride. *Journal of AOAC International,* **75**, pp. 949-953.

DABEKA, R.W., MCKENZIE, A.D., CONACHER, H.B.S., AND KIRKPATRICK, B.S., 1982. Determination of fluoride in Canadian infant foods and calculation of fluoride intake by infants. *Canadian Journal of Public Health,* **73**, pp. 188-191.

DAS, A., GUPTA, R.D., FRIEDMAN, J., PRADHAN, M.M., MOHAPATRA, C.C. and SANDHIBIGRAHA, D., 2013. Community perceptions on malaria and care-seeking practices in endemic Indian settings: policy implications for the malaria control programme. *Malaria Journal,* **12**(1), p. 39.

DASGUPTA, K., DA COSTA, D., PILLAY, S., DE CIVITA, M., GOUGEON, R., LEONG, A., BACON, S., STOTLAND, S., CHETTY, V.T. and GARFIELD, N., 2013. Strategies to optimize participation in diabetes prevention programs following gestational diabetes: a focus group study. *PloS one,* **8**(7), pp. e67878.

DE ALMEIDA, B.S., DA SILVA CARDOSO, V.E. and BUZALAF, M.A.R., 2007. Fluoride ingestion from toothpaste and diet in 1-to 3-year-old Brazilian children. *Community Dentistry and Oral Epidemiology,* **35**(1), pp. 53-63.

DE BERKER, D., ANDRÉ, J. and BARAN, R., 2007. Nail biology and nail science. *International Journal of Cosmetic Science,* **29**(4), pp. 241-275.

DE SOUZA, C.F., LIMA, J.F. JR, ADRIANO, M.S., DE CARVALHO, F.G., FORTE, F.D., DE FARIAS OLIVEIRA, R., SILVA, A.P. and SAMPAIO, F.C., 2012. Assessment of groundwater quality in a region of endemic fluorosis in the northeast of Brazil. *Environmental Monitoring and Assessment,* **185**(6), pp. 4735-4743.

DEAN, H.T., ARNOLD JR, F.A., JAY, P. and KNUTSON, J.W., 1950. Studies on mass control of dental caries through fluoridation of the public water supply. *Public Health Reports*, **65**(83), pp. 1403-1408.

DEAN, H.T. and ARNOLD, JR. F.A. AND ELVOVE, E., 1942. Domestic Water and Dental Caries: V. Additional Studies of the Relation of Fluoride Domestic Waters to Dental Caries Experience in 4,425 White Children, Aged 12 to 14 Years, of 13 Cities in 4 States. *Public Health Reports,* **57**(32), pp. 1155-1179.

DENBESTEN, P.K. and THARIANI, H., 1992. Biological mechanisms of fluorosis and level and timing of systemic exposure to fluoride with respect to fluorosis. *Journal of Dental Research*, *71*(5), pp.1238-1243.

DHAR, V. and BHATNAGAR, M., 2009. Physiology and toxicity of fluoride. *Indian Journal of Dental Research,* **20**(3), pp. 350-355.

DIBAL, H.U. AND LAR, U.A., 2005. Preliminary survey of fluoride concentrations in the groundwaters of Kaltungo town and environs. Gombe state, northeastern Nigeria: Water quality and health implications. *Journal of Environmental Sciences,* **9**(2), pp. 41-52.

DIBAL, H.U., LEKMANG, I.C. AND LAR, U.A., 2008. Dental fluorosis from drinking water consumption in Lantang town Plateau state, Nigeria. *Continental Journal of Earth Sciences,* **3**, pp. 77-82.

DIBAL, H., SCHOENEICH, K., GARBA, I., LAR, U. and BALA, E., 2012. Occurrence of fluoride in the drinking waters of Langtang area, north central Nigeria. *Health,* **4(**11**)**, pp. 1116-1126.

DIBAL, H.U., SCHOENEICH, K., GARBA, I., LAR, U. and BALA, E., 2012. Overview of fluoride distribution in major aquifer units of northern Nigeria. *Health,* **4**(12), pp. 1287-1294.

DILLIP, A., ALBA, S., MSHANA, C., HETZEL, M.W., LENGELER, C., MAYUMANA, I., SCHULZE, A., MSHINDA, H., WEISS, M.G. and OBRIST, B., 2012. Acceptability–a neglected dimension of access to health care: findings from a study on childhood convulsions in rural Tanzania. *BMC Health Services Research,* **12**(1), pp. 113-124.

DONOHUE, J.M., DUKE, T., OPRESKO, D., WATSON, A. AND TOMKINS, B., 2010. Fluoride: Exposure and relative source contribution analysis. *Washington, DC: US Environmental Protection Agency US Environmental Protection Agency*.

DOWELL, T.B., 1981. The use of toothpaste in infancy. *British Dental Journal,* **150**(9), pp. 247-249.

DRUMMOND, H.E., GHOSH, S., FERGUSON, A., BRACKENRIDGE, D. AND TIPLADY, B., 1995. Electronic quality of life questionnaires: a comparison of pen-based electronic questionnaires with conventional paper in a gastrointestinal study. *Quality of life research*, *4*(1), pp.21-26.

DUCKWORTH, R.M., KNOOP, D.T. and STEPHEN, K.W., 1991. Effect of mouthrinsing after toothbrushing with a fluoride dentifrice on human salivary fluoride levels. *Caries Research,* **25**(4), pp. 287-291.

DUCKWORTH, R.M., HORAY, C., HUNTINGTON, E. and MEHTA, V., 2009. Effects of flossing and rinsing with a fluoridated mouthwash after brushing with a fluoridated toothpaste on salivary fluoride clearance. *Caries Research,* **43**(5), pp. 387-390.

DUCKWORTH, R.M. and JONES, S., 2015. On the relationship between the rate of salivary flow and salivary fluoride clearance. *Caries Research,* **49**(2), pp. 141-146.

EBLE, D.M., DEATON, T.G., WILSON, F.C. and BAWDEN, J.W., 1992. Fluoride concentrations in human and rat bone. *Journal of Public Health Dentistry,* **52**(5), pp. 288-291.

EFSA, 2005. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Fluoride. *EFSA Journal,* **192**, pp. 1-65.

EFSA PANEL ON DIETETIC PRODUCTS, NUTRITION AND ALLERGIES (NDA), 2013. Scientific opinion on dietary reference values for fluoride. *EFSA Journal,* **11**(8), pp. 3332-3346.

EGGLETON, M.G., 1942. The diuretic action of alcohol in man. *The Journal of Physiology,* **101**(2), pp. 172-191.

EKSTRAND, J. and EHRNEBO, M., 1979. Influence of milk products on fluoride bioavailability in man. *European Journal of Clinical Pharmacology,* **16**(3), pp. 211-215.

EKSTRAND, J., HARDELL, L. and SPAK, C., 1984. Fluoride balance studies on infants in a 1-ppm-water-fluoride area. *Caries Research,* **18**(1), pp. 87-92.

EKSTRAND, J., 1977. A micromethod for the determination of fluoride in blood plasma and saliva. *Calcified Tissue Research,* **23**(1), pp. 225-228.

EKSTRAND, J., EHRNEBO, M. and BORÉUS, L.O., 1978. Fluoride bioavailability after intravenous and oral administration: importance of renal clearance and urine flow. *Clinical Pharmacology & Therapeutics,* **23**(3), pp. 329-337.

EKSTRAND, J., FOMON, S.J., ZIEGLER, E.E. and NELSON, S.E., 1994. Fluoride pharmacokinetics in infancy. *Pediatric Research,* **35**(2), pp. 157-163.

EKSTRAND, J. and SPAK, C., 1990. Fluoride pharmacokinetics: its implications in the fluoride treatment of osteoporosis. *Journal of Bone and Mineral Research,* **5**(S1), pp. S53-S61.

EKSTRAND, J., 1989. Fluoride intake in early infancy. *The Journal of nutrition,* **119**(12 Suppl), pp. 1856-1860.

EL-NADEEF, M.A.I. and HONKALA, E., 1998. Fluorosis in relation to fluoride levels in water in central Nigeria. *Community dentistry and oral epidemiology,* **26**(1), pp. 26-30.

ELSAIR, J., MERAD, R., DENINE, R., KHELFAT, K., AOUL, M., ASSUNKUMAR, B., REGGABI, M., AZZOUZ, M., HAMROUR, S. and ALAMIR, B., 1982. Fluoride content of urine, blood, nails and hair in endemic skeletal fluorosis. *Fluoride,* **15**(1), pp. 43-47.

ENABULELE, O. and AWUNOR, S.N., 2016. Typhoid fever in a Tertiary Hospital in Nigeria: Another look at the Widal agglutination test as a preferred option for diagnosis. *Nigerian Medical journal,* **57**(3), pp. 145-149.

ERICKSON, J.D., OAKLEY, G.P., FLYNT, J.W. AND HAY, S., 1976. Water fluoridation and congenital malformations: no association. *The Journal of the American Dental Association*, *93*(5), pp.981-984.

EVERETT, E.T., 2011. Fluoride's effects on the formation of teeth and bones, and the influence of genetics. *Journal of Dental Research,* **90**(5), pp. 552-560.

EVM (EXPERT GROUP ON VITAMINS AND MINERALS), 2001. *Review of fluoride.* Food Standards Agency London.

FABIO, C.S., 2006. Biomarkers of exposure to fluoride. *Journal of Applied Oral Science,* **14**(Special iss), pp. 1-3.

FAUL, F., ERDFELDER, E., LANG, A. and BUCHNER, A., 2007. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods,* **39**(2), pp. 175-191.

FEATHERSTONE, J.D., 1999. Prevention and reversal of dental caries: role of low level fluoride. *Community dentistry and oral epidemiology,* **27**(1), pp. 31-40.

FEWTRELL, L., CHILTON, J., DAHI, E., FAWELL, J., MAGARA, Y., and BAILEY, K., 2006. Fluoride in drinking-water. World Health Organization, Geneva.

FISHER, P.A., GUNNAR, M.R., DOZIER, M., BRUCE, J. and PEARS, K.C., 2006. Effects of therapeutic interventions for foster children on behavioral problems, caregiver attachment, and stress regulatory neural systems. *Annals of the New York Academy of Sciences,* **1094**(1), pp. 215-225.

FOMON, S.J., EKSTRAND, J. and ZIEGLER, E.E., 2000. Fluoride Intake and Prevalence of Dental Fluorosis: Trends in Fluoride Intake with Special Attention to Infants. *Journal of Public Health Dentistry,* **60**(3), pp. 131-139.

FOMON S.J., EKSTRAND, J., 1996. Fluoride intake. In: FEJERSKOV O, EKSTRAND J, BURT BA, ed, *Fluoride in Dentistry.* Red edn. Munksgaard, København, pp. 40-52.

FORSMAN, B. and ERICSSON, Y., 1973. Fluoride absorption from swallowed fluoride toothpaste. *Community Dentistry and Oral Epidemiology,* **1**(3), pp. 115-120.

FRANCO, Á.M., MARTIGNON, S., SALDARRIAGA, A., GONZÁLEZ, M.C., ARBELÁEZ, M.I., OCAMPO, A., LUNA, L.M., MARTÍNEZ-MIER, E. and VILLA, A.E., 2005. Total fluoride intake in children aged 22–35 months in four Colombian cities. *Community Dentistry and Oral Epidemiology,* **33**(1), pp. 1-8.

FRANZMAN, M.R., LEVY, S.M., WARREN, J.J. and BROFFITT, B., 2004. Tooth-brushing and dentifrice use among children ages 6 to 60 months. *Pediatric Dentistry,* **26**(1), pp. 87-92.

FREEZE, R.A. and LEHR, J.H., 2009. *The Fluoride Wars: How a Modest Public Health Measure Became America's Longest Running Political Melodrama.* John Wiley & Sons. New Jersey, US.

FRENI, S.C., 1994. Exposure to high fluoride concentrations in drinking water is associated with decreased birth rates. *Journal of Toxicology and Environmental Health, Part A Current Issues*, *42*(1), pp.109-121.

FUKUSHIMA, R., PESSA, J.P. and SAMPAIO, F.C. AND BUZALAF, M.A.R, 2011. Factors associated with fluoride concentrations in whole and parotid ductal saliva. *Caries Research,* **45**, pp. 568-573.

FUKUSHIMA, R., RIGOLIZZO, D.S., MAIA, L.P., SAMPAIO, F.C. and LAURIS, J.R. AND BUZALAF, M.A.R., 2009. Environmental and individual factors associated with nail fluoride concentration. *Caries Research,* **43**, pp. 147-154.

FUKUSHIMA, T., AFKHAM, A., KUROSAWA, S., TANABE, T., YAMAMOTO, H. and SEKIGUCHI, J., 2006. A new D,L-endopeptidase gene product, YojL (renamed CwlS), plays a role in cell separation with LytE and LytF in Bacillus subtilis. *Journal of Bacteriology,* **188**(15), pp. 5541-5550.

FURLANI, T.D.A., WHITFORD, G.M., GRANJEIRO, J.M. and BUZALAF, M.A.R., 2001. Effect of storage time on detectable fluoride in rat nails. *Revista da Faculdade de Odontologia de Bauru,* **9**(3/4), pp. 179-181.

GASEM, M.H., SMITS, H.L., GORIS, M.G. and DOLMANS, W.M., 2002. Evaluation of a simple and rapid dipstick assay for the diagnosis of typhoid fever in Indonesia. *Journal of Medical Microbiology,* **51**(2), pp. 173-177.

GERSOVITZ, M., MADDEN, J.P. and SMICIKLAS-WRIGHT, H., 1978. Validity of the 24-hr. dietary recall and seven-day record for group comparisons. *Journal of the American Dietetic Association,* **73**(1), pp. 48-55.

GOODSON, M.L. and VERNON, B.G., 2004. A study of public opinion on the use of tissue samples from living subjects for clinical research. *Journal of Clinical Pathology,* **57**(2), pp. 135-138.

GRIFFITH, J., DUNCAN, R.C. and HULKA, B.S., 1989. Biochemical and biological markers: implications for epidemiologic studies. *Archives of Environmental Health: An International Journal,* **44**(6), pp. 375-381.

GRIJALVA-HARO, M.I., BARBA-LEYVA, M.E. and LABORÍN-ALVAREZ, A., 2001. Ingestión y excreción de fluoruros en niños de Hermosillo, Sonora, México. *salud pública de méxico,* **43**(2), pp. 127-134.

GROBLER, S., ROSSOUW, R. and KOTZE, T.V.W., 1998. A comparison of fluoride release from various dental materials. *Journal of Dentistry,* **26**(3), pp. 259-265.

GUHA-CHOWDHURY, N., DRUMMOND, B. and SMILLIE, A., 1996. Total fluoride intake in children aged 3 to 4 years—A longitudinal study. *Journal of Dental Research,* **75**(7), pp. 1451-1457.

GUO, Z.Y., HE, Y.H. AND ZHU, Q.X., 2001. Study on neurobehavioral function of workers occupationally exposed to fluoride. *Industrial Health and Occupational Diseases-Beijing-*, *27*(6), pp.346-348.

GUSKIT, R., 2010. Major and trace element distribution in natural waters and soils in Panyam Volcanic Province-Health Implication. *MSc Thesis, Department of Geology and Mining, University of Jos, Nigeria*.

GUY, W.S., TAVES, D.R., & BREY, W.S., JR, 1976. Organic fluoro-compounds in human plasma: Prevalence and characterization. In: R. FULLER, ed, *Biochemistry involving carbon-fluorine bonds.* Symposium Series 28 edn. American Chemical Society, pp. 117-134.

HAFTENBERGER, M., VIERGUTZ, G., NEUMEISTER, V. and HETZER, G., 2001. Total fluoride intake and urinary excretion in German children aged 3-6 years. *Caries Research,* **35**(6), pp. 451-7.

HALDIMANN, M. and ZIMMERLI, B., 1993. Evaluation of ashing procedures for the gas chromatographic determination of fluoride in biological material. *Analytica Chimica Acta,* **282**(3), pp. 589-601.

HALL, R., 1968. Observations on the distribution and determination of fluorine compounds in biological materials, including soils. *Analyst,* **93**(1108), pp. 461-468.

HAMASHA, A.A., LEVY, S.M., BROFFITT, B. and WARREN, J.J., 2005. Patterns of dietary fluoride supplement use in children from birth to 96 months of age. *Journal of Public Health Dentistry,* **65**(1), pp. 7-13.

HARKEY, M. and HENDERSON, G., 1988. Hair Analysis for Drugs of Abuse: A Critical Review of the Technology. *Report submitted to California Department of Alcohol and Drug Programs.* Winters, CA: Henderson-Harkey, Inc,

HATTAB, F.N., 1989. Analytical methods for the determination of various forms of fluoride in toothpastes. *Journal of Dentistry,* **17**(2), pp. 77-83.

HECTOR, F., MARIA, A.A., MARGERET, P., ANTHONY, V. and FATIMA, R., 2009. Fluoride intake and urinary fluoride excretion in children attending a daycare in Maracay, Aragua State, Venezuela. *Journal of Dentistry and Oral Hygiene,* **1**(13), pp. 27-35.

HEDMAN, J., SJÖMAN, R., SJÖSTRÖM, I. and TWETMAN, S., 2006. Fluoride Concentration in Saliva after Consumption of a Dinner Meal Prepared with Fluoridated Salt. *Caries Research,* **40**(2), pp. 158-62.

HELLWIG, E. and LENNON, A.M., 2004. Systemic versus topical fluoride. *Caries Research,* **38**(3), pp. 258-262.

HOEYER, K., JENSEN, A. and OLEJAZ, M., 2015. Transplantation as an abstract good: practising deliberate ignorance in deceased organ donation in Denmark. *Sociology of Health & Illness,* **37**(4), pp. 578-593.

HONG, L., LEVY, S.M., BROFFITT, B., WARREN, J.J., KANELLIS, M.J., WEFEL, J.S. and DAWSON, D.V., 2006. Timing of fluoride intake in relation to development of fluorosis on maxillary central incisors. *Community Dentistry and Oral Epidemiology*, *34*(4), pp.299-309.

IGBE, M.A. and ADEBAMOWO, C.A., 2012. Qualitative study of knowledge and attitudes to biobanking among lay persons in Nigeria. *BMC medical ethics,* **13**(1), pp. 27-36.

IKENISHI, R. and KITAGAWA, T., 1988. Gas Chromatographic Method for the Determination of Fluoride Ion in Biological Samples. II.: Stability of Fluorine-Containing Drugs and Compounds in Human Plasma. *Chemical and Pharmaceutical Bulletin,* **36**(2), pp. 810-814.

IKENISHI, R., KITAGAWA, T., NISHIUCHI, M., TAKEHARA, K., YAMADA, H., NISHINO, I., UMEDA, T., IWATANI, K., NAKAGAWA, Y. and SAWAI, M., 1988. Gas Chromatographic Method for the Determination of Fluoride Ion in Biological Samples. I.: Fluoride Level in Monkey. *Chemical and Pharmaceutical Bulletin,* **36**(2), pp. 662-669.

IPCS (INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY), 2002. *Fluorides. Environmental Health Criteria 227.* Geneva, Switzerland: World Health Organization.

IPCS INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY, 1984. *Fluorine and fluorides.* www.inchem.org/documents/ehc/ehc/ehc36.htm edn. Geneva: World Health Organization.

ISHIGURO, K., NAKAGAKI, H., TSUBOI, S., NARITA, N., KATO, K., LI, J., KAMEI, H., YOSHIOKA, I., MIYAUCHI, K. and HOSOE, H., 1993. Distribution of fluoride in cortical bone of human rib. *Calcified Tissue International,* **52**(4), pp. 278-282.

Japan International Cooperation Agency, 2013. The challenge of inproving sanitation in sub-saharan Africa-finding a way to change people's behavior and build a community without water borne diseases. Available at: https://www.jica.go.jp/english/news/focus_on/ticad_v/articles/article24.html#anc01 (Accessed: 22 February 2018).

JARYUM, S., 2005. *Geogenic concentration of some major and trace element in the groundwaters of Jos east local government area.* MSc edn. Jos Nigeria: University of Jos, Geology, Mining Department.

JEANDEL, C., LAPICQUE, F., NETTER, P., BANNWARTH, B., MONOT, C., GILLET, P., PAYAN, E., GUILLAUME, M. and CUNY, G., 1992. Effect of age on the disposition of sodium fluoride. *European Journal of Clinical Pharmacology,* **43**(3), pp. 295-297.

JHA, S.K., MISHRA, V.K., SHARMA, D.K. and DAMODARAN, T., 2011. Fluoride in the environment and its metabolism in humans. *Reviews of Environmental Contamination and Toxicology,* **211**, pp. 121-142.

JOHANSSON, I., HALLMANS, G., WIKMAN, Å., BIESSY, C., RIBOLI, E. and KAAKS, R., 2002. Validation and calibration of food-frequency questionnaire measurements in the Northern Sweden Health and Disease cohort. *Public Health Nutrition,* **5**(3), pp. 487-496.

JOHNSON, T., ZHANG, W., BICHARD, S. and KAYE, B., 2008. Shut up and listen: The influence of selective exposure to blogs and political websites on political tolerance, *Annual Meeting of the Midwest Association for Public Opinion Research, Chicago, IL* 2008.

JOHNSSON, L., HANSSON, M.G., ERIKSSON, S. and HELGESSON, G., 2008. Patients' refusal to consent to storage and use of samples in Swedish biobanks: cross sectional study. *BMJ,* **337**, pp. a345.

JOSHI, D. and MEAKIN, R., 2017. Views and attitudes towards blood donation: a qualitative investigation of Indian non-donors living in England. *BMJ open,* **7**(10), pp. e018279-2017-018279.

KAKABADSE, G., MANOHIN, B., BATHER, J., WELLER, E. and WOODBRIDGE, P., 1971. Decomposition and the determination of fluorine in biological materials. *Nature,* **229**(5287), pp. 626-627.

KAVANAGH, P., FARAGO, M., THORNTON, I., GOESSLER, W., KUEHNELT, D., SCHLAGENHAUFEN, C. and IRGOLIC, K., 1998. Urinary arsenic species in Devon and Cornwall residents, UK. A pilot study. *Analyst,* **123**(1), pp. 27-29.

KAVANAGH, D. and RENEHAN, J., 1998. Fluoride in tea--its dental significance: a review. *Journal of the Irish Dental Association,* **44**(4), pp. 100-105.

KETLEY, C.E., COCHRAN, J.A., HOLBROOK, W.P., SANCHES, L., VAN LOVEREN, C., OILA, A. and O'MULLANE, D.M., 2004. Urinary fluoride excretion by preschool children in six European countries. *Community dentistry and oral epidemiology,* **32**(s1), pp. 62-68.

KETLEY, C.E. and LENNON, M.A., 2001. Determination of fluoride intake from urinary fluoride excretion data in children drinking fluoridated school milk. *Caries research,* **35**(4), pp. 252-257.

KHAN, A., MOOLA, M.H. and CLEATON-JONES, P., 2005. Global trends in dental fluorosis from 1980 to 2000: a systematic review. *South African Dental Journal,* **60**(10), pp. 418-421.

KHANDARE, A., RAO, G. and LAKSHMAIAH, N., 2002. Effect of tamarind ingestion on fluoride excretion in humans. *European Journal of Clinical Nutrition,* **56**(1), pp. 82-85.

KIMURA, T., MORITA, M., KINOSHITA, T., TSUNEISHI, M., AKAGI, T., YAMASHITA, F. and WATANABE, T., 2001. Fluoride intake from food and drink in Japanese children aged 1-6 years. *Caries Research,* **35**(1), pp. 47-49.

KINTZ, P., 2004. Value of hair analysis in postmortem toxicology. *Forensic Science International,* **142**(2-3), pp. 127-134.

KNIGHT, H.G., FURR, A.K. and PARKINSON, T., 1977. Determination of fluorine by neutron activation analysis. *Analytical Chemistry,* **49**(11), pp. 1507-1510.

KOBAYASHI, C.A.N., LEITE, A.D.L., SILVA, T.L.D., SANTOS, L.D.D., NOGUEIRA, F.C.S., SANTOS, K.S., OLIVEIRA, R.C.D., PALMA, M.S., DOMONT, G.B. and BUZALAF, M.A.R., 2011. Proteomic analysis of urine in rats chronically exposed to fluoride. *Journal of Biochemical and Molecular Toxicology,* **25**(1), pp. 8-14.

KOKOT, Z. AND DRZEWIECKI, D., 2000. Fluoride levels in hair of exposed and unexposed populations in Poland. *Fluoride,* **33**(4), pp. 196-204.

KONGSVED, S.M., BASNOV, M., HOLM-CHRISTENSEN, K. and HJOLLUND, N.H., 2007. Response rate and completeness of questionnaires: a randomized study of Internet versus paper-and-pencil versions. *Journal of Medical Internet Research*, *9*(3).

KONO, K., 1997. Biological monitoring of fluoride-exposed workers especially for health care of elderly workers. *Fluoride,* **30**(1), pp. 61-63.

KONO, K., YOSHIDA, Y., WATANABE, M., ORITA, Y., DOTE, T. and BESSHO, Y., 1993. Urine, serum and hair monitoring of hydrofluoric acid workers. *International Archives of Occupational and Environmental Health,* **65**(1), pp. S95-S98.

KONO, K., YOSHIDA, Y., WATANABE, M., TANIOKA, Y., ORITA, Y., DOTE, T., BESSHO, Y., TAKAHASHI, Y., YOSHIDA, J. and SUMI, Y., 1992. Serum fluoride as an indicator of occupational hydrofluoric acid exposure. *International Archives of Occupational and Environmental Health,* **64**(5), pp. 343-346.

KONO, K., YOSHIDA, Y., WATANABE, M., WATANABE, H., INOUE, S., MURAO, M. and DOI, K., 1990. Elemental analysis of hair among hydrofluoric acid exposed workers. *International archives of occupational and environmental health,* **62**(1), pp. 85-88.

KONO, K., YOSHIDA, Y., YAMAGATA, H., WATANABE, M., SHIBUYA, Y. and DOI, K., 1987. Urinary fluoride monitoring of industrial hydrofluoric acid exposure. *Environmental Research,* **42**(2), pp. 415-420.

KUMAR, A. and SUSHEELA, A.K., 1995. Effects of chronic fluoride toxicity on the morphology of ductus epididymis and the maturation of spermatozoa of rabbit. *International Journal of Experimental Pathology,* **76**(1), pp. 1-11.

LAKSHMI AV AND LAKSHMAIAH N, 1999. Effect of different cereal based diet on fluoride retention in rats. National seminar on fluoride contamination, fluoride and defluoridation techniques, Udaypur, India, February 25-27 1999.

LAL, H., ZOHOORI, F.V., OMID, N., VALENTINE, R. and MAGUIRE, A., 2014. The fluoride contents of commercially-available soya milks in the UK. *British Dental Journal,* **217**(4), pp. E8-E8.

LAR, U., DIBAL, H., DASPAN, R. and JARYUM, S., 2007. Fluoride occurrence in the surface and groundwaters of Fobur area of Jos East LGA, Plateau State. *Journal of Environmental Sciences,* **11**(2), pp. 99-105.

LECH, T., 2011. Fatal cases of acute suicidal sodium and accidental zinc fluorosilicate poisoning. Review of acute intoxications due to fluoride compounds. *Forensic Science International,* **206**(1), pp. e20-e24.

LEVY, S.M., HILLIS, S.L., WARREN, J.J., BROFFITT, B.A., MAHBUBUL ISLAM, A., WEFEL, J.S. and KANELLIS, M.J., 2002. Primary tooth fluorosis and fluoride intake during the first year of life. *Community Dentistry and Oral Epidemiology,* **30**(4), pp. 286-295.

LEVY, S.M., KIRITSY, M., SLAGER, S., WARREN, J. and KOHOUT, F.J., 1997. Patterns of fluoride dentifrice use among infants. *Pediatric Pentistry,* **19**, pp. 50-55.

LEVY, S.M., MCGRADY, J.A., BHURIDEJ, P., WARREN, J.J., HEILMAN, J.R. and WEFEL, J.S., 2000. Factors affecting dentifrice use and ingestion among a sample of US preschoolers. *Pediatric Pentistry,* **22**(5), pp. 389-394.

LEVY, S.M., WARREN, J.J., DAVIS, C.S., KIRCHNER, H.L., KANELLIS, M.J. and WEFEL, J.S., 2001. Patterns of fluoride intake from birth to 36 months. *Journal of public health dentistry,* **61**(2), pp. 70-77.

LEVY, F.M., BASTOS, J.R. and BUZALAF, M.A., 2004. Nails as biomarkers of fluoride in children of fluoridated communities. *Journal of Dentistry for Children,* **71**(2), pp. 121-125.

LEVY, S.M., KOHOUT, F.J., GUHA-CHOWDHURY, N., KIRITSY, M.C., HEILMAN, J.R. and WEFEL, J.S., 1995. Infants' fluoride intake from drinking water alone, and from water added to formula, beverages, and food. *Journal of Dental Research,* **74**(7), pp. 1399-1407.

LEVY, S.M., WARREN, J.J., BROFFITT, B., HILLIS, S.L. and KANELLIS, M.J., 2003. Fluoride, beverages and dental caries in the primary dentition. *Caries Research,* **37**(3), pp. 157-165.

LEWIS, C., CLOTWORTHY, M., HILTON, S., MAGEE, C., ROBERTSON, M.J., STUBBINS, L.J. and CORFIELD, J., 2013. Public views on the donation and use of human biological samples in biomedical research: a mixed methods study. *BMJ Open,* **3**(8), pp. 10.1136/bmjopen-2013-003056.

LI, Y., LI, X. and WEI, S., 1994. The effects of high fluoride intake on child mental work capacity and preliminary investigation into mechanisms involved. *Journal of West China University Medical Science,* **25**(2), pp. 188-191.

LI, J., YAO, L., SHAO, Q.L. AND WU, C.Y., 2004. Effects of high-fluoride on neonatal neurobehavioural development. *Chinese Journal of Epidemiology*, *23*(5), pp.463-465.

LIDDELL, K. and HALL, A., 2005. Beyond Bristol and Alder Hey: the future regulation of human tissue. *Medical Law Review,* **13**(2), pp. 170-223.

LIMA, Y.B. and CURY, J.A., 2003. Seasonal variation of fluoride intake by children in a subtropical region. *Caries Research,* **37**(5), pp. 335-338.

LIMA-ARSATI, Y.B.O., MARTINS, C.C., ROCHA, L.A. and CURY, J.A., 2010. Fingernail may not be a reliable biomarker of fluoride body burden from dentifrice. *Brazilian Dental Journal,* **21**(2), pp. 91-97.

LINHARES, D.P.S., GARCIA, P.V., AMARAL, L., FERREIRA, T., CURY, J.A., VIEIRA, W. and DOS SANTOS RODRIGUES, A., 2016. Sensitivity of two biomarkers for biomonitoring exposure to fluoride in children and women: A study in a volcanic area. *Chemosphere,* **155**, pp. 614-620.

LIU, H., CHEN, J., HUNG, H., HSIAO, S., HUANG, S. and CHEN, H., 2011. Urinary fluoride concentration in children with disabilities following long-term fluoride tablet ingestion. *Research in Developmental Disabilities,* **32**(6), pp. 2441-2448.

MABELYA, L., PALENSTEIN HELDERMAN, W.V., HOF, M.V. and KÖNIG, K., 1997. Dental fluorosis and the use of a high fluoride-containing trona tenderizer (magadi). *Community Dentistry and Oral Epidemiology,* **25**(2), pp. 170-176.

MAGUIIRE, A., WALLS, R., STEEN, N., TEASDALE, L., LANDES, D., OMID, N.,MOYNIHAN, P. and ZOHOORI, F.V., 2013. Urinary fluoride excretion in 6-7 year olds ingesting milk containing 0.5 or 0.9mg fluoride. *Caries Research,* **47**, pp. 291-298.

MAGUIRE, A., OMID, N., ABUHALOOB, L., MOYNIHAN, P.J. and ZOHOORI, F.V., 2012. Fluoride content of Ready-to-Feed (RTF) infant food and drinks in the UK. *Community dentistry and oral epidemiology,* **40**(1), pp. 26-36.

MAGUIRE, A., ZOHOURI, F., HINDMARCH, P., HATTS, J. and MOYNIHAN, P., 2007. Fluoride intake and urinary excretion in 6-to 7-year-old children living in optimally, sub-optimally and non-fluoridated areas. *Community dentistry and oral epidemiology,* **35**(6), pp. 479-488.

MAGUIRE, A. and ZOHOORI, F.V., 2013. Fluoride balance in infants and young children in the UK and its clinical relevance for the dental team. *British dental journal,* **214**(11), pp. 587-93.

MAGUIRE, A., ZOHOURI, F.V., MATHERS, J.C., STEEN, I.N., HINDMARCH, P.N. and MOYNIHAN, P.J., 2005. Bioavailability of fluoride in drinking water: a human experimental study. *Journal of dental research,* **84**(11), pp. 989-993.

MAHESHWARI, U.R., MCDONALD, J.T., SCHNEIDER, V.S., BRUNETTI, A.J., LEYBIN, L., NEWBRUN, E. and HODGE, H.C., 1981. Fluoride balance studies in ambulatory healthy men with and without fluoride supplements. *The American Journal of Clinical Nutrition,* **34**(12), pp. 2679-2684.

MALDE, M.K., BJORVATN, K. and JULSHAMN, K., 2001. Determination of fluoride in food by the use of alkali fusion and fluoride ion-selective electrode. *Food Chemistry,* **73**(3), pp. 373-379.

MANDINIC, Z., CURCIC, M., ANTONIJEVIC, B., CAREVIC, M., MANDIC, J. and DJUKIC-COSIC, D. AND LEKIC, C.P., 2010. Fluoride in drinking water and dental fluorosis. *Science of The Total Environment,* **408**, pp. 3507-3512.

MANDINIC, Z., CURCIC, M., ANTONIJEVIC, B., LEKIC, C.P. and CAREVIC, M., 2009. Relationship between fluoride intake in Serbian children living in two areas with different natural levels of fluorides and occurrence of dental fluorosis. *Food and Chemical Toxicology,* **47**(6), pp. 1080-1084.

MANDINIC, Z., CURCIC, M., ANTONIJEVIC, B., NEDELJKOVIC, M. and CAREVIC, M., 2007. Fluoride levels in hair samples—Biomarker of exposure. *Toxicology Letters,* **172(Suppl.)**, p. S119.

MARGETTS, B.M. and NELSON, M., 1997. *Design Concepts in Nutritional Epidemiology.* OUP, Oxford.

MARSHALL, P.L., 2007. *Ethical challenges in study design and informed consent for health research in resource-poor settings.* World Health Organization, Geneva.

MARSHALL, T.A., GILMORE, J.M.E., BROFFITT, B., STUMBO, P.J. and LEVY, S.M., 2008. Relative validity of the Iowa Fluoride Study targeted nutrient semi-quantitative questionnaire and the block kids' food questionnaire for estimating beverage, calcium, and vitamin D intakes by children. *Journal of the American Dietetic Association,* **108**(3), pp. 465-472.

MARTHALER, T.M., 1999. *Monitoring real fluoride excretion in community preventive programmes on oral health.* Geneva: World Health Organization.

MARTHALER, T.M., BINDER-FUCHS, M., BAEZ, R. and MENGHINI, G., 2000. Urinary fluoride excretion in Swiss children aged 3 and 4 consuming fluoridated domestic salt. *Schweizerische Monatsschrift für Zahnmedizin,* **110**(6), pp. 89-596.

MARTHALER, T. and PETERSEN, P., 2005. Salt fluoridation—an alternative in automatic prevention of dental caries. *International Dental Journal,* **55**(6), pp. 351-358.

MARTÍNEZ-MIER, E.A., 2012. Fluoride: Its metabolism, toxicity, and role in dental health. *Journal of Evidence-Based Complementary & Alternative Medicine,* **17**(1), pp. 28-32.

MARTINEZ-MIER, E.A. and SOTO-ROJAS, A.E., 2010. Differences in exposure and biological markers of fluoride among White and African American children. *Journal of public health dentistry,* **70**(3), pp. 234-240.

MARTINEZ-MIER, E.A., CURY, J.A., HEILMAN, J.R., KATZ, B.P., LEVY, S.M., LI, Y., MAGUIRE, A., MARGINEDA, J., O'MULLANE, D., PHANTUMVANIT, P., SOTO-ROJAS, A.E., STOOKEY, G.K., VILLA, A., WEFEL, J.S., WHELTON, H., WHITFORD, G.M., ZERO, D.T., ZHANG, W. and ZOHOURI, V., 2011. Development of gold standard ion-selective electrode-based methods for fluoride analysis. *Caries research,* **45**(1), pp. 3-12.

MARTIN-MORENO, J.M., BOYLE, P., GORGOJO, L., MAISONNEUVE, P., FERNANDEZ-RODRIGUEZ, J.C., SALVINI, S. and WILLETT, W.C., 1993. Development and validation of a food frequency questionnaire in Spain. *International journal of epidemiology,* **22**(3), pp. 512-519.

MARTIN-MORENO, J.M. and GORGOJO, L., 2007. Assessment of dietary intake at the population level through individual questionnaires: methodological shadows and lights. *Revista Española de Salud Pública,* **81**(5), pp. 507-518.

MCCLURE, F.J., 1943. Observations on induced caries in rats: IV. Inhibiting effect of fluoride ingested post-eruptively and prior to the caries producing diet. *Journal of dental research,* **22**(1), pp. 37-43.

MCDONNELL, S.T., O'MULLANE, D., CRONIN, M., MACCORMAC, C. and KIRK, J., 2004. Relevant factors when considering fingernail clippings as a fluoride biomarker. *Community dental health,* **21**(1), pp. 19-24.

MCGRADY, M.G., ELLWOOD, R.P., SRISILAPANAN, P., KORWANICH, N., WORTHINGTON, H.V. and PRETTY, I.A, 2012. Dental fluorosis in populations of Chiang Mai, Thailand with different fluoride exposures-Paper 1: assessing fluorosis risk, predictors of fluorosis and the potential role of food preparation. *BMC Oral Health,* **12**(1), pp. 16-28.

MCGRADY, M.G., ELLWOOD, R.P., MAGUIRE, A., GOODWIN, M., BOOTHMAN, N. and PRETTY, I.A., 2012. The association between social deprivation and the prevalence and severity of dental caries and fluorosis in populations with and without water fluoridation. *BMC Public Health,* **12**, pp. 1122-1139.

MCKAY, F., 1952. The study of mottled enamel (dental fluorosis). *Journal of the American Dental Association,* **44**(2), pp. 133-137.

MEDICAL RESEARCH COUNCIL (UK), 2002. *Water fluoridation and health*. Medical Research Council working group report.

MEHTA, A., 2013. Biomarkers of fluoride exposure in human body. *Indian Journal of Dentistry,* **4**(4), pp. 207-210.

MELLO, M.M. and WOLF, L.E., 2010. The Havasupai Indian tribe case—lessons for research involving stored biologic samples. *New England Journal of Medicine,* **363**(3), pp. 204-207.

MESSER, H. and OPHAUG, R., 1993. Influence of gastric acidity on fluoride absorption in rats. *Journal of Dental Research,* **72**(3), pp. 619-622.

MCKINNEY, H.L., 2000. Fluoride in hair. *Fluoride,* **33**(4), pp. 149-150.

MIZIARA, A.P.B., PHILIPPI, S.T., LEVY, F.M. and BUZALAF, M.A.R., 2009. Fluoride ingestion from food items and dentifrice in 2–6-year-old Brazilian children living in a fluoridated area using a semiquantitative food frequency questionnaire. *Community Dentistry and Oral Epidemiology,* **37**(4), pp. 305-315.

MOKGOBI, M., 2014. Understanding traditional African healing. *African Journal for Physical Health Education, Recreation and Dance,* **20**(Supplement 2), pp. 24-34.

MOLLER, I.J., 1982. Fluorides and dental fluorosis. *International Dental Journal,* **32**(2), pp. 135-147.

MOLYNEUX, D.H. and NANTULYA, V.M., 2004. Linking disease control programmes in rural Africa: a pro-poor strategy to reach Abuja targets and millennium development goals. *BMJ,* **328**(7448), pp. 1129-1132.

MOODLEY, K., SIBANDA, N., FEBRUARY, K. and ROSSOUW, T., 2014. "It's my blood": ethical complexities in the use, storage and export of biological samples: perspectives from South African research participants. *BMC medical ethics,* **15**(1), pp. 4-11.

MORÁN FAGÚNDEZ, L.J., RIVERA TORRES, A., GONZÁLEZ SÁNCHEZ, M.E., DE TORRES AURED, MARI LOURDES, LÓPEZ-PARDO MARTÍNEZ, M. and IRLES ROCAMORA, J.A., 2015. Historical overview of diet assessment and food consumption surveys in Spain: assessment methods and applications. *Nutrición Hospitalaria,* **31**(Suppl. 3), 22-28.

MORÁN FAGÚNDEZ, L.J., RIVERA TORRES, A., GONZÁLEZ SÁNCHEZ, M.E., DE TORRES AURED, MARI LOURDES, PÉREZ RODRIGO, C. and IRLES ROCAMORA, J.A., 2015. Diet history: Method and applications. *Nutricion hospitalaria,* **31**(Suppl. 3), 57-61.

MORRELL, B., LIPWORTH, W., AXLER, R., KERRIDGE, I. and LITTLE, M., 2011. Cancer as rubbish: donation of tumor tissue for research. *Qualitative health research,* **21**(1), pp. 75-84.

MURRAY, J.J., RUGG-GUNN, A.J. and JENKINS, G.N., eds, 1991. *Fluoride in Caries Prevention.* 3rd edn. 1991: Wright/Butterworth-Heinemann. Boston.

MUSSALI-GALANTE, P., TOVAR-SÁNCHEZ, E., VALVERDE, M. and ROJAS DEL CASTILLO, E., 2013. Biomarkers of exposure for assessing environmental metal pollution: from molecules to ecosystems. *Revista Internacional de Contaminación Ambiental,* **29**(1), pp. 117-140.

NACCACHE, H., SIMARD, P.L., TRAHAN, L., BRODEUR, J., DEMERS, M., LACHAPELLE, D. and BERNARD, P., 1992. Factors affecting the ingestion of fluoride dentifrice by children. *Journal of public health dentistry,* **52**(4), pp. 222-226.

National Cancer Institute, 2017. Dietary assessment primer: food frequency questionnaire at a glance. Available at: https://dietassessmentprimer.cancer.gov/profiles/questionnaire/ (Accessed: 22 November 2017).

NATIONAL RESEARCH COUNCIL, 1993. *Health effects of ingested fluoride*. National Academies Press.

NATIONAL RESEARCH COUNCIL, 2007. *Fluoride in drinking water: a scientific review of EPA's standards.* National Academies Press. Washington, US.

NEDELJKOVIĆ, M., ANTONIJEVIĆ, B. and MATOVIĆ, V., 1991. Simplified sample preparation for fluoride determination in biological material. *Analyst,* **116**(5), pp. 477-478.

NÉMETH, L. and ZSÖGÖN, É., 1989. Occupational skeletal fluorosis. *Baillière's Clinical Rheumatology,* **3**(1), pp. 81-88.

NEWMAN, M.G. and CARRANZA JR, F.A., 1985. *Fluoride-coated dental floss*. *U.S. Patent No. 4,548,219*. Washington, DC: U.S. Patent and Trademark Office.

NEWTON, S., DOKU, V., GEISSLER, W., ASANTE, K.P. and COUSENS, S., 2009. Drawing blood from young children: lessons learned from a trial in Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **103**(5), pp. 497-499.

NGWU, B.A. and AGBO, J.A., 2003. Typhoid fever: clinical diagnosis versus laboratory confirmation. *Nigerian Journal of Medicine,* **12**(4), pp. 187-192.

NIELSEN, J. and DANGERFIELD, A., 1955. Use of ion exchange: resins for determination of atmospheric fluorides. *Arch.Indust.Health,* **11**(1), pp. 61-65.

NNYANZI, L.A., 2012. The national child measurement programme: Its value and impact. PhD Thesis, Teesside University.

NOHNO, K., ZOHOORI, F.V. and MAGUIRE, A., 2011. Fluoride intake of Japanese infants from infant milk formula. *Caries Research,* **45**(5), pp. 486-493.

NORDFALK, F., OLEJAZ, M., JENSEN, A.M., SKOVGAARD, L.L. and HOEYER, K., 2016. From motivation to acceptability: a survey of public attitudes towards organ donation in Denmark. *Transplantation Research,* **5**(1), pp. 5-13.

NUFFIELD COUNCIL ON BIOETHICS, 1995. *Human tissue: Ethical and legal issues.* Nuffield Council on Bioethics. London.

OFFICE OF NATIONAL STATISTICS, 2011. Census. Available at:https://www.ons.gov.uk/census/2011census (Accessed: 5 May 2016).

O MULLANE, D., BAEZ, R., JONES, S., LENNON, M., PETERSEN, P.E., RUGG-GUNN, A., WHELTON, H. and WHITFORD, G., 2016. Fluoride and oral health. *Community Dental Health,* **33**(2), pp. 69-99.

OLIVEBY, A., LAGERLÖF, F. and EKSTRAND, J. and DAWES, C., 1989. Influence of flow rate, pH, and plasma fluoride concentrations on fluoride concentration in human parotid saliva. *Archives of Oral Biology,* **34**(3), pp. 191-194.

OLIVEBY, A., WEETMAN, D., GEDDES, D. and LAGERLÖF, F., 1990. The effect of salivary clearance of sucrose and fluoride on human dental plaque acidogenicity. *Archives of Oral Biology,* **35**(11), pp. 907-911.

OMID, N., ZOHOORI, F.V., MAGUIRE, A, 2013. Assessment of direct and diffusion methods of fluoride analysis of soya-milks. *Caries Research,* **47**(1), p. 478.

OMID, N., MAGUIRE, A., O'HARE, W. and ZOHOORI, F.V., 2015. Estimation of daily dietary fluoride intake: 3-d food diary v. 2-d duplicate plate. *British Journal of Nutrition,* **114**(12), pp. 2103-2109.

OMID, N., 2012. The assessment level of fluoride intake/exposure using "3-day dietary diary" & "2-day duplicate" methods. PhD thesis Teesside University.

ONWUJEKWE, O., DIKE, N., UZOCHUKWU, B. and EZEOKE, O., 2010. Informal payments for healthcare: differences in expenditures from consumers and providers perspectives for treatment of malaria in Nigeria. *Health Policy,* **96**(1), pp. 72-79.

OPHAUG, R., SINGER, L. and HARLAND, B., 1985. Dietary fluoride intake of 6-month and 2-year-old children in four dietary regions of the United States. *The American Journal of Clinical Nutrition,* **42**(4), pp. 701-707.

OPHAUG, R.H., SINGER, L. and HARLAND, B.F., 1980. Estimated fluoride intake of 6-month-old infants in four dietary regions of the United States. *The American Journal of Clinical Nutrition,* **33**(2), pp. 324-327.

OSUJP, O., LEAKE, J., CHIPMAN, M., NIKIFORUK, G., LOCKER, D. and LEVINE, N., 1988. Risk factors for dental fluorosis in a fluoridated community. *Journal of Dental Research,* **67**(12), pp. 1488-1492.

PAKHOMOV, G.N., IVANOVA, K., MOLLER, I.J. and VRABCHEVA, M., 1995. Dental caries-reducing effects of a milk fluoridation project in Bulgaria. *Journal of Public Health Dentistry,* **55**(4), pp. 234-237.

PAKHOMOV, G.N., KOLESNIK, A.G., SHAMSHEVA, A.A., KUZ'MIN, E.M. and STEPANOVA, I.A., 2005. Milk fluoridization efficacy in a controlled study and dental caries experience dynamics in conditions of wide availability of local F-containing means. *Stomatologiia,* **84**(4), pp. 37-42.

PANG, D.T.Y., PHILLIPS, C.L. and BAWDEN, J.W., 1992. Fluoride intake from beverage consumption in a sample of North Carolina children. *Journal of Dental Research,* **71**(7), pp. 1382-1388.

PARIMI, N., VISWANATH, V. and KASHYAP, B. AND PATIL, P.U., 2013. Hair as biomarker of fluoride exposure in a fluoride endemic area and low fluoridated area. *International Journal of Trichology,* **5**(3), pp. 148-150.

PARNELL, W., SCRAGG, R., WILSON, N., SCHAAF, D. and FITZGERALD, E., 2003. NZ food NZ children. *Key Results of the 2002 National Children's Nutrition Survey.Wellington: Ministry of Health* .

PARRY, C.M., HOA, N.T., DIEP, T.S., WAIN, J., CHINH, N.T., VINH, H., HIEN, T.T., WHITE, N.J. and FARRAR, J.J., 1999. Value of a single-tube widal test in diagnosis of typhoid fever in Vietnam. *Journal of Clinical Microbiology,* **37**(9), pp. 2882-2886.

PAUSTENBACH, D. and GALBRAITH, D., 2006. Biomonitoring: is body burden relevant to public health? *Regulatory Toxicology and Pharmacology,* **44**(3), pp. 249-261.

PENTZ, R.D., BILLOT, L. and WENDLER, D., 2006. Research on stored biological samples: views of African American and White American cancer patients. *American Journal of Medical Genetics Part A,* **140**(7), pp. 733-739.

PESSAN, J.P. and BUZALAF, M.R., 2011. Historical and recent biological markers of exposure to fluoride. In, Buzalaf, M.A.R. ed., Fluoride and the Oral Environment. *Monographs in oral science,* **22**, pp. 52-65.

PESSAN, J.P., PIN, M.L., MARTINHON, C.C., DE SILVA, S.M., GRANJEIRO, J.M. and BUZALAF, M.A., 2005. Analysis of fingernails and urine as biomarkers of fluoride exposure from dentifrice and varnish in 4- to 7-year-old children. *Caries Research,* **39**(5), pp. 363-370.

PESSAN, J.P., SILVA, S.M., LAURIS, J.R., SAMPAIO, F.C., WHITFORD, G.M. and BUZALAF, M.A., 2008. Fluoride uptake by plaque from water and from dentifrice. *Journal of Dental Research,* **87**(5), pp. 461-465.

PETERSEN, P.E. and LENNON, M.A., 2004. Effective use of fluorides for the prevention of dental caries in the 21st century: the WHO approach. *Community Dentistry and Oral Epidemiology,* **32**(5), pp. 319-321.

PETERSSON, L., LUDVIGSSON, N., ULLBRO, C., GLEERUP, A. and KOCH, G., 1987. Fluoride clearance of whole saliva in young school children after topical application. *Swedish Dental Journal,* **11**(3), pp. 95-101.

PHILIPPI, S.T., CRUZ, A.T.R. and COLUCCI, A.C.A., 2003. Food guide pyramid for young children 2 to 3 years old. *Revista de Nutrição,* **16**(1), pp. 5-19.

PHILIPPI, S.T., LATTERZA, A.R., CRUZ, A.T.R. and RIBEIRO, L.C., 1999. Adapted food pyramid: a guide for a right food choice. *Revista de Nutrição,* **12**(1), pp. 65-80.

PIKHOLZ, C., SWINBURN, B. and METCALF, P., 2004. Under-reporting of energy intake in the 1997 National Nutrition Survey. *New Zealand Medical Journal,* **117**(1202), pp. 1-11.

PITTS, N., DUCKWORTH, R.M., MARSH, P., MUTTI, B., PARNELL, C. AND ZERO, D., 2012. Post-brushing rinsing for the control of dental caries: exploration of the available evidence to establish what advice we should give our patients. *British dental journal*, *212*(7), p.315.

PONIKVAR, M., 2008. Chapter 12 - Exposure of Humans to Fluorine and Its Assessment. In: A. TRESSAUD, ed, *Fluorine and Health.* Amsterdam: Elsevier, pp. 487-549.

PREEDY, V.R., 2015. *Fluorine: Chemistry, Analysis, Function and Effects.* Royal Society of Chemistry. London UK.

QUENTIN, K., 1967. Colorimetric Methods for Fluoride Determination. *Caries Research,* **1**(4), pp. 288-294.

RANGO, T., VENGOSH, A., JEULAND, M., WHITFORD, G.M. and TEKLE-HAIMANOT, R., 2017. Biomarkers of chronic fluoride exposure in groundwater in a highly exposed population. *Science of the Total Environment,* **596**, pp. 1-11.

RANGO, T., VENGOSH, A., JEULAND, M., TEKLE-HAIMANOT, R., WEINTHAL, E., KRAVCHENKO, J., PAUL, C. and MCCORNICK, P., 2014. Fluoride exposure from groundwater as reflected by urinary fluoride and children's dental fluorosis in the Main Ethiopian Rift Valley. *Science of the Total Environment,* **496**, pp. 188-197.

RGNDWM, 1993. *Prevention and control of fluorosis, Health Aspects*. Ministry of Rural Development, Rajiv Gandhi National Drinking Water Mission, Govt. of India, New Delhi, 1, 89p.

RIZEK, R.L. and PAO, E.M., 1990. Dietary intake methodology I. USDA surveys and supporting research. *The Journal of Nutrition,* **120**(suppl_11), pp. 1525-1529.

ROCHA-AMADOR, D., NAVARRO, M., TREJO-ACEVEDO, A., CARRIZALES, L., PÉREZ-MALDONADO, I., DÍAZ-BARRIGA, F. and CALDERÓN, J., 2009. Use of the Rey-Osterrieth Complex Figure Test for neurotoxicity evaluation of mixtures in children. *Neurotoxicology*, *30*(6), pp.1149-1154.

ROJAS-SANCHEZ, F., KELLY, S.A., DRAKE, K.M., ECKERT, G.J., STOOKEY, G.K. and DUNIPACE, A.J., 1999. Fluoride intake from foods, beverages and dentifrice by young children in communities with negligibly and optimally fluoridated water: a pilot study. *Community Dentistry and Oral Epidemiology,* **27**(4), pp. 288-297.

RÖLLA, G. and EKSTRAND, J., 1996. Fluoride in oral fluids and dental plaque. *Fluoride in dentistry.2nd edn. Copenhagen: Munksgaard,* pp. 215-229.

RUGG-GUNN, A., NUNN, J., EKANAYAKE, L., SAPARAMADU, K. and WRIGHT, W., 1993. Urinary fluoride excretion in 4-year-old children in Sri Lanka and England. *Caries research,* **27**(6), pp. 478-483.

RUGG-GUNN, A.J., VILLA, A.E. and BUZALAF, M.R., 2011. Contemporary biological markers of exposure to fluoride. In, Buzalaf, M.A.R. ed., Fluoride and the Oral Environment. *Monographs in Oral Science,* **22**, pp. 37-51.

SAITOH, M., 1969. Rate of hair growth. *Advances in Biology of Skin,* **9**, pp. 183-202.

SANDERSON, C., 2013. Fluoride best applied topically through toothpaste. *Kai Tiaki Nursing New Zealand,* **19**(7), p. 3.

SANKHALA, S., HARSHWAL, R., PALIWAL, P. and AGARWALC, A., 2014. Toe nails as a biomarker of chronic fluoride exposure secondary to high water fluoride content in areas with endemic fluorosis. *Fluoride,* **47**(3), pp. 235-240.

SARA, R. and WÄNNINEN, E., 1975. Separation and determination of fluoride by diffusion with hexamethyldisiloxane and use of a fluoride-sensitive electrode. *Talanta,* **22**(12), pp. 1033-1036.

SCHAMSCHULA, R.G., SUGÁR, E., UN, P.S., TÓTH, K., BARMES, D.E. and ADKINS, B.L., 1985. Physiological indicators of fluoride exposure and utilization: an epidemiological study. *Community Dentistry and Oral Epidemiology,* **13**(2), pp. 104-117.

SCHER (SCIENTIFIC COMMITTEE ON HEALTH AND ENVIRONMENTAL RISKS) OF THE EUROPEAN COMMISSION, 2010. *Critical review of any new evidence on the hazard profile, health effects, and human exposure to fluoride and the fluoridating agents of drinking water.* Brussels: European Commission.

SCHIFFL, H. and BINSWANGER, U., 1982. Renal handling of fluoride in healthy man. *Kidney and Blood Pressure Research,* **5**(4), pp. 192-196.

SCHMIDT, C. and FUNKE, U., 1984. Renale Fluoridausscheidung nach Belastung mit Schwarzem Tee. *Zeitschrift für ärztliche Fortbildung,* **78**, pp. 364-367.

SCHMIDT, C. and LEUSCHKE, W., 1990. Fluoride content in fingernails of individuals with and without chronic fluoride exposure. *Fluoride,* **23**(2), pp. 79-82.

SCHOUR, I., MASSLER, M., 1944. *Development of the human dentition. American Dental Association*, **28**, pp. 1153-1160.

SEKHON, M., CARTWRIGHT, M. and FRANCIS, J.J., 2017. Acceptability of healthcare interventions: an overview of reviews and development of a theoretical framework. *BMC Health Services Research,* **17**(1), pp. 113-124.

SHEN, Y.W. and TAVES, D.R., 1974. Fluoride concentrations in the human placenta and maternal and cord blood. *American Journal of Obstetrics and Gynecology,* **119**(2), pp. 205-207.

SHULMAN, J.D., LALUMANDIER, J.A. and GRABENSTEIN, J.D., 1995. The average daily dose of fluoride: a model based on fluid consumption. *Pediatric Dentistry,* **17**(1), pp. 13-18.

SIDANI, S., EPSTEIN, D.R., BOOTZIN, R.R., MORITZ, P. and MIRANDA, J., 2009. Assessment of preferences for treatment: validation of a measure. *Research in Nursing & Health,* **32**(4), pp. 419-431.

SINGER, L. and ARMSTRONG, W., 1965. Determination of fluoride: Procedure based upon diffusion of hydrogen fluoride. *Analytical Biochemistry,* **10**(3), pp. 495-500.

SINGER, L. and OPHAUG, R.H., 1986. Determination of fluoride in foods. *Journal of Agricultural and Food Chemistry,* **34**(3), pp. 510-513.

SLATER, B., PHILIPPI, S., FISBERG, R. and LATORRE, M., 2003. Validation of a semi-quantitative adolescent food frequency questionnaire applied at a public school in São Paulo, Brazil. *European journal of clinical nutrition,* **57**(5), pp. 629-635.

SLOOFF, W., EERENS, H.C., JANUS, J.A. AND ROS, J.P.M., 1988. *Basis Document Fluoriden.* Bilthoven, Netherlands: RIVM.

SPAK, C.J., BERG, U. and EKSTRAND, J., 1985. Renal clearance of fluoride in children and adolescents. *Pediatrics,* **75**(3), pp. 575-579.

SPATE, V., MORRIS, J., BASKETT, C., MASON, M., REAMS, C., HUNTER, D. and WILLETT, W., 1994. Determination of fluoride in human nails via cyclic instrumental neutron activation analysis. *Journal of Radioanalytical and Nuclear Chemistry,* **179**(1), pp. 27-33.

SPENCER, H., OSIS, D., WIATROWSKI, E. and SAMACHSON, J., 1970. Availability of fluoride from fish protein concentrate and from sodium fluoride in man. *The Journal of Nutrition,* **100**(12), pp. 1415-1424.

SPENCER, H., LEWIN, I., WISTROWSKI, E. and SAMACHSON, J., 1970. Fluoride metabolism in man. *The American Journal of Medicine,* **49**(6), pp. 807-813.

SPITTLE, B., 1993. Allergy and hypersensitivity to fluoride. *Fluoride*, *26*, pp.267-267.

STEPHEN, K.W., BOYLE, I.T., CAMPBELL, D., MCNEE, S. and BOYLE, P., 1984. Five-year double-blind fluoridated milk study in Scotland. *Community Dentistry and Oral Epidemiology,* **12**(4), pp. 223-229.

STOLARSKA, K., CZARNOWSKI, W., URBANSKA, B. and KRECHNIAK, J., 2000. Fluoride in hair as an indicator of exposure to fluorine compounds. *Fluoride,* **33**(4), pp. 174-181.

STRATTON, R.J., STUBBS, R.J., HUGHES, D., KING, N., BLUNDELL, J.E. AND ELIA, M., 1998. Comparison of the traditional paper visual analogue scale questionnaire with an Apple Newton electronic appetite rating system (EARS) in free living subjects feeding ad libitum. *European journal of clinical nutrition*, *52*(10), p.737.

SUSHEELA, A., BHATNAGAR, M. and VIG, K., 2005. Excess fluoride ingestion and thyroid hormone derangements in children living in Delhi, India. *Fluoride,* **38**(2), pp. 98-108.

SUSHEELA, A.K., MONDAL, N.K. and SINGH, A., 2013. Exposure to fluoride in smelter workers in a primary aluminum industry in India. *The International Journal of Occupational and Environmental Medicine,* **4**(2 April), pp. 61-72.

SZÉKELY, M., FAZAKAS, Z., BALOGH-SAMARGHITAN, V., BÁNÓCZY, J. and TÓTH, Z., 2010. Fluoride excretion in whole saliva and urine after intake of fluoridated milk with or without breakfast in young adults. *Oral Health Dental Management,* **9**(4), pp. 207-213.

SZPUNAR, S.M. and BURT, B.A., 1992. Evaluation of appropriate use of dietary fluoride supplements in the US. *Community Dentistry and Oral Epidemiology,* **20**(3), pp. 148-154.

TAVES, D.R., 1983. Dietary intake of fluoride ashed (total fluoride) v. unashed (inorganic fluoride) analysis of individual foods. *British Journal of Nutrition,* **49**(03), pp. 295-301.

TAVES, D.R., 1968. Separation of fluoride by rapid diffusion using hexamethyldisiloxane. *Talanta,* **15**(9), pp. 969-974.

TAYLOR, M.L., MACONNACHIE, E., FRANK, K., BOYDE, A. and JONES, S.J., 1990. The effect of fluoride on the resorption of dentine by osteoclasts in vitro. *Journal of Bone and Mineral Research,* **5**(S), pp. 121-130.

THE ROYAL COLLEGE OF PATHOLOGISTS, 2002-last update, Human bodies, human choices: response from the Royal College of Pathologists. Available: www.rcpath.org [01/10, 2018].

TINDANA, P., BULL, S., AMENGA-ETEGO, L., DE VRIES, J., ABORIGO, R., KORAM, K., KWIATKOWSKI, D. and PARKER, M., 2012. Seeking consent to genetic and genomic research in a rural Ghanaian setting: a qualitative study of the MalariaGEN experience. *BMC medical ethics,* **13**(1), pp. 15-27.

TOMOMATSU, T., SUZUKI, J., NAKAZAWA, K. AND KIMURA, Y., 1976. Hygienic study on fluorine. (III) Analysis of fluorine in foods and estimation of dietary fluorine intake. *Annual Report of the Tokyo Metropolitan Research Laboratory of Public Health,* **27**, pp. 174-178.

TOMORI, T., KOGA, H., MAKI, Y. and TAKAESU, Y., 2004. Fluoride analysis of foods for infants and estimation of daily fluoride intake. *The Bulletin of Tokyo Dental College,* **45**(1), pp. 19-32.

TORRA, M., RODAMILANS, M. and CORBELLA, J., 1998. Serum and urine fluoride concentration: relationships to age, sex and renal function in a non-fluoridated population. *Science of the total environment,* **220**(1), pp. 81-85.

TOSTI, A., DANIEL, R., PIRACCINI, B.M. and IORIZZO, M., 2009. *Color Atlas of Nails.* Springer Science & Business Media. Berlin, Germany.

TOTH, M., GINTNER, Z. and BANOCZY, J., 2005. The effect of ingested fluoride administrated in salt, milk and tablet on salivary and urinary fluoride concentrations. *Fluoride,* **38**, pp. 199-204.

TREWEEK, S. and ZWARENSTEIN, M., 2009. Making trials matter: pragmatic and explanatory trials and the problem of applicability. *Trials,* **10**(1), pp. 37-46.

TSUNODA, K., FUJIWARA, K. and FUWA, K., 1977. Subnanogram fluorine determination by aluminum monofluoride molecular absorption spectrometry. *Analytical Chemistry,* **49**(13), pp. 2035-2039.

TYLENDA, C.A., 2003. *Toxicological profile for fluorides, hydrogen fluoride, and fluorine.* Agency for Toxic Substances and Disease Registry. Atlanta US.

US PUBLIC HEALTH SERVICE, 1991. Review of fluoride benefits and risks. *Washington, DC: Department of Health and Human Services*.

VAN DEN BERG, M.H., OVERBEEK, A., VAN DER PAL, H.J., VERSLUYS, A.B., BRESTERS, D., VAN LEEUWEN, F.E., LAMBALK, C.B., KASPERS, G.J. and VAN DULMEN-DEN BROEDER, E., 2011. Using web-based and paper-based questionnaires for collecting data on fertility issues among female childhood cancer survivors: differences in response characteristics. *Journal of Medical Internet Research*, *13*(3).

VAN LOVEREN, C., KETLEY, C.E., COCHRAN, J.A., DUCKWORTH, R.M. and O'MULLANE, D.M., 2004. Fluoride ingestion from toothpaste: fluoride recovered from the toothbrush, the expectorate and the after-brush rinses. *Community Dentistry and Oral Epidemiology,* **32**(s1), pp. 54-61.

VAN LOVEREN, C., 2004. Sugar alcohols: what is the evidence for caries-preventive and caries-therapeutic effects? *Caries Research,* **38**(3), pp. 286-293.

VAN SCHALKWYK, G., DE VRIES, J. and MOODLEY, K., 2012. " It's for a good cause, isn't it?"-Exploring views of South African TB research participants on sample storage and re-use. *BMC Medical Ethics,* **13**(1), pp. 19-25.

VENKATESWARLU, P., 1990. Evaluation of analytical methods for fluorine in biological and related materials. *Journal of dental research,* **69**(2_suppl), pp. 514-521.

VENKATESWARLU, P. and NARAYANA, R., 1954. Investigations on the removal of fluoride from water: factors governing the adsorptions of fluoride by magnesium oxide. *Indian Journal of Medical Research,* **42**(1), pp. 135-140.

VENKATESWARLU, P., SINGER, L. and ARMSTRONG, W., 1971. Determination of ionic (plus ionizable) fluoride in biological fluids: Procedure Based on Adsorption of Fluoride Ion on Calcium Phosphate. *Analytical Biochemistry,* **42**(2), pp. 350-359.

VENKATESWARLU, P., 1992. Separation of fluoride (from fluoroelastomers) by diffusion in test tubes. *Analytical Chemistry,* **64**(4), pp. 346-349.

VENKATESWARLU, P., 1994. Determination of fluorine in biological materials: a review. *Advances in Dental Research,* **8**(1), pp. 80-86.

VERMEULEN, E., SCHMIDT, M.K., AARONSON, N.K., KUENEN, M., VAN DER VALK, P., SIETSES, C., VAN DEN TOL, P. and VAN LEEUWEN, F.E., 2009. Opt-out plus, the patients' choice: preferences of cancer patients concerning information and consent regimen for future research with biological samples archived in the context of treatment. *Journal of clinical pathology,* **62**(3), pp. 275-278.

VIEIRA, A., HANCOCK, R., LIMEBACK, H., MAIA, R. and GRYNPAS, M., 2004. Is fluoride concentration in dentin and enamel a good indicator of dental fluorosis? *Journal of dental research,* **83**(1), pp. 76-80.

VIEIRA, A., HANOCOCK, R., EGGERTSSON, H., EVERETT, E. and GRYNPAS, M., 2005. Tooth quality in dental fluorosis. *Calcified Tissue International,* **76**(1), pp. 17-25.

VIEIRA, A., MOUSNY, M., MAIA, R., HANCOCK, R., EVERETT, E. and GRYNPAS, M., 2005. Assessment of teeth as biomarkers for skeletal fluoride exposure. *Osteoporosis International,* **16**(12), pp. 1576-1582.

VILLA, A., ANABALON, M. and CABEZAS, L., 2000. The fractional urinary fluoride excretion in young children undern stable fluoride intake conditions. *Community Dentistry & Oral Epidemiology,* **28**, pp. 344-345.

VILLA, A., SALAZAR, G., ANABALÓN, M. and CABEZAS, L., 1999. Estimation of the fraction of an ingested dose of fluoride excreted through urine in pre-school children. *Community Dentistry and Oral Epidemiology,* **27**(4), pp. 305-312.

VILLA, A., ANABALON, M., CABEZAS, L. and RUGG-GUNN, A., 2008. Fractional urinary fluoride excretion of young female adults during the diurnal and nocturnal periods. *Caries Research,* **42**(4), pp. 275-281.

VILLA, A., ANABALON, M., ZOHOURI, V., MAGUIRE, A., FRANCO, A.M. and RUGG-GUNN, A., 2010. Relationships between fluoride intake, urinary fluoride excretion and fluoride retention in children and adults: an analysis of available data. *Caries Research,* **44**(1), pp. 60-68.

VILLA, A., CABEZAS, L., ANABALON, M. and GARZA, E., 2004. The fractional urinary fluoride excretion of adolescents and adults under customary fluoride intake conditions, in a community with 0.6-mg F/L in its drinking water. *Community Dental Health,* **21**(1), pp. 11-18.

VINE, M.F., 1994. Biological markers: their use in quantitative assessments. *Advances in Dental Research,* **8**(1), pp. 92-99.

VISWANATHAN, G., JASWANTH, A. and GOPALAKRISHNAN, S., 2009. Mapping of fluoride endemic areas and assessment of fluoride exposure. *Science of the Total Environment,* **407**(5), pp. 1579-1587.

WADE, M. and YAMAMURA, S.S., 1965. Microdetermination of fluoride using an improved distillation procedure. *Analytical Chemistry,* **37**(10), pp. 1276-1278.

WALDBOTT, G.L., 1978. *Health effects of environmental pollutants.* The CV Mosby Company, 11830 Westline Industrial Drive, St, Louis, Missouri 63141, USA.

WALDBOTT, G.L., 1963. Fluoride in food. *The American Journal of Clinical Nutrition,* **12**, pp. 455-462.

WALTERS, C.B., SHERLOCK, J.C., EVANS, W.H. and READ, J.I., 1983. Dietary intake of fluoride in the United Kingdom and fluoride content of some foodstuffs. *Journal of the Science of Food and Agriculture,* **34**(5), pp. 523-528.

WANG, B., ZHENG, B., ZHAI, C., YU, G. and LIU, X., 2004. Relationship between fluorine in drinking water and dental health of residents in some large cities in China. *Environment International,* **30**(8), pp. 1067-1073.

WARNAKULASURIYA, K., BALASURIYA, S., PERERA, P. and PEIRIS, L., 1992. Determining optimal levels of fluoride in drinking water for hot, dry climates-a case study in Sri Lanka. *Community Dentistry and Oral Epidemiology,* **20**(6), pp. 364-367.

WARREN, J.J., LEVY, S.A. and KANELLIS, M.J., 2002. Dental caries in the primary dentition: assessing prevalence of cavitated and noncavitated lesions. *Journal of Public Health Dentistry,* **62**(2), pp. 109-114.

WARREN, J.J., LEVY, S.M. and KANELLIS, M.J., 2001. Prevalence of dental fluorosis in the primary dentition. *Journal of Public Health Dentistry,* **61**(2), pp. 87-91.

WATANABE, M., KONO, K., ORITA, Y., YDOTE, T., USUDA, K., TAKAHASHI, Y., YOSHIDA, Y. and DOI, K., 1994. Influence of dietary fluoride intake on urinary fluoride concentration and evaluation of corrected levels in spot urine, *Proceedings of the XXth Conference of the International Society for Fluoride Research, Beijing, China. Beijing: Ministry of Public Health of People's Republic of China* 1994, pp. 246-247.

WATERHOUSE, C., TAVES, D. and MUNZER, A., 1980. Serum inorganic fluoride: changes related to previous fluoride intake, renal function and bone resorption. *Clinical Science,* **58**(2), pp. 145-152.

WAZIRI, M., MUSA, U. and HATI, S.S., 2012. Assessment of Fluoride Concentrations in Surface Waters and Groundwater Sources in Northeastern Nigeria. *Assessment,* **2**(2), pp. 67-72.

WEATHERELL, J., DEUTSCH, D., ROBINSON, C. and HALLSWORTH, A., 1977. Assimilation of fluoride by enamel throughout the life of the tooth. *Caries Research,* **11**(Suppl. 1), pp. 85-115.

WEATHERELL, J., ROBINSON, C. and HALLSWORTH, A., 1972. Changes in the fluoride concentration of the labial enamel surface with age. *Caries Research,* **6**(4), pp. 312-324.

WELCOME, M.O., 2011. The Nigerian health care system: Need for integrating adequate medical intelligence and surveillance systems. *Journal of Pharmacy & Bioallied Sciences,* **3**(4), pp. 470-478.

WENDLER, D., 2006. One-time general consent for research on biological samples: is it compatible with the health insurance portability and accountability act? *Archives of Internal Medicine,* **166**(14), pp. 1449-1452.

WENDLER, D., PACE, C., TALISUNA, A.O., MAISO, F., GRADY, C. and EMANUEL, E., 2005. Research on stored biological samples: the views of Ugandans. *IRB*, **27**(2), pp. 1-5.

WHITFORD, G.M., 2011. Acute toxicity of ingested fluoride. In, Buzalaf, M.A.R. ed., Fluoride and the Oral Environment. *Monograph in Oral SCience,* **22**, pp. 68-80.

WHITFORD, G.M., ed, 1996. *The metabolism and toxicity of fluoride.* 2 edn. Basel: Karger.

WHITFORD, G.M. and PASHLEY, D.H., 1984. Fluoride absorption: the influence of gastric acidity. *Calcified Tissue International,* **36**(1), pp. 302-307.

WHITFORD, G.M., PASHLEY, D.H. and REYNOLDS, K.E., 1979. Fluoride tissue distribution: short-term kinetics. *American Journal of Physiology,* **236**(2), pp. F141-F148.

WHITFORD, G., 1997. Determinants and mechanisms of enamel fluorosis. *Ciba Foundation Symposium,* **205**, pp. 226-241.

WHITFORD, G., SAMPAIO, F., PINTO, C., MARIA, A., CARDOSO, V. and BUZALAF, M., 2008. Pharmacokinetics of ingested fluoride: lack of effect of chemical compound. *Archives of Oral Biology,* **53**(11), pp. 1037-1041.

WHITFORD, G.M., 2005a. Fluoride metabolism when added to salt. *Schweizer Monatsschrift für Zahnmedizin,* **115**(8), pp. 675-678.

WHITFORD, G.M., 2005b. Monitoring fluoride exposure with fingernail clippings. *Schweiz Monatsschr Zahnmed,* **115**, pp. 685-689.

WHITFORD, G.M., 1989. The metabolism and toxicity of fluoride. *Monographs in oral science,* **13**.

WHITFORD, G.M., ALLMANN, D.W. and SHAHED, A.R., 1987. Topical fluorides: effects on physiologic and biochemical processes. *Journal of Dental Research,* **66**(5), pp. 1072-1078.

WHITFORD, G.M., SAMPAIO, F.C., ARNEBERG, P. and VON DER FEHR, F.R., 1999. Fingernail fluoride: A method for monitoring fluoride exposure. *Caries Research,* **33**, pp. 462-467.

WHITFORD, G.M., THOMAS, J.E. and ADAIR, S.M., 1999. Fluoride in whole saliva, parotid ductal saliva and plasma in children. *Archives of Oral Biology,* **44**(10), pp. 785-788.

WHO, 1994. *Fluorides and oral health.* Geneva, Switzerland: World Health Organisation.

WIATROWSKI, E., KRAMER, L., OSIS, D. and SPENCER, H., 1975. Dietary fluoride intake of infants. *Pediatrics,* **55**(4), pp. 517-522.

WILLARD, H. and WINTER, O.B., 1933. Volumetric method for determination of fluorine. *Industrial & Engineering Chemistry Analytical Edition,* **5**(1), pp. 7-10.

WILLETT, W.C., STAMPFER, M.J., UNDERWOOD, B.A., SPEIZER, F.E., ROSNER, B. and HENNEKENS, C.H., 1983. Validation of a dietary questionnaire with plasma carotenoid and α-tocopherol levels. *The American Journal of Clinical Nutrition,* **38**(4), pp. 631-639.

WILLETT, W., 2012. Food frequency methods. *In Nutritional Epidemiology.* 2nd edn. New York: Oxford University Press.

WILLISON, D.J., SCHWARTZ, L., ABELSON, J., CHARLES, C., SWINTON, M., NORTHRUP, D. and THABANE, L., 2007. Alternatives to project-specific consent for access to personal information for health research: what is the opinion of the Canadian public? *Journal of the American Medical Informatics Association,* **14**(6), pp. 706-712.

WILLISON, D.J., 2007. Data protection and the promotion of health research: if the laws are not the problem, then what is? *Healthcare policy = Politiques de sante,* **2**(3), pp. 39-43.

WONGDEM, J.G., ADERINOKUN, G.A., UBOM, G.A., SRIDHAR, M.K. and SELKUR, S., 2001. Dental fluorosis and fluoride mapping in Langtang town, Nigeria. *African Journal of Medicine and Medical Sciences,* **30**(1-2), pp. 31-34.

WORLD HEALTH ORGANISATION (WHO), 1984. *Fluorine and fluorides (Environmental Health Criteria 36).* Geneva: International Programme on Chemical Safety.

WORLD HEALTH ORGANIZATION, 2014. *Basic methods for assessing renal fluoride excretion in community prevention programmes for oral health.* World Health Organization, Geneva.

WORLD HEALTH ORGANIZATION, 2006. *Fluoride in drinking-water.* 2006: World Health Organization, Geneva.

Marthaler TM., 1999. *Monitoring of Renal Fluoride Excretion in Community Preventive Programmes on Oral Health*, *Geneva,* World Health Organization.

WORLD HEALTH ORGANIZATION, 1996. *Guidelines for Drinking Water Quality (Vol 2).* 2nd edn. Geneva: WHO.

XU, J., YUAN, X. and LANG, P., 1997. Determination of catalase activity and catalase inhibition by ultraviolet spectrophotometry. *Chin. Environ. Chem.,* **16**, pp. 73-76.

YADAV, A.K., KAUSHIK, C.P., HARITASH, A.K., SINGH, B., RAGHUVANSHI, S.P. and KANSAL, A., 2007. Determination of exposure and probable ingestion of fluoride through tea, toothpaste, tobacco and pan masala. *Journal of Hazardous Materials,* **142**(1–2), pp. 77-80.

YAEMSIRI, S., HOU, N., SLINING, M. and HE, K., 2010. Growth rate of human fingernails and toenails in healthy American young adults. *Journal of the European Academy of Dermatology and Venereology,* **24**(4), pp. 420-423.

YAZDI, S.M., SHARIFIAN, A., DEHGHANI-BESHNE, M., MOMENI, V.R. AND AMINIAN, O., 2011. Effects of fluoride on psychomotor performance and memory of aluminum potroom workers. *Fluoride*, *44*(3), pp.158-162.

YODER, K.M., MABELYA, L., ROBISON, V.A., DUNIPACE, A.J., BRIZENDINE, E.J. and STOOKEY, G.K., 1998. Severe dental fluorosis in a Tanzanian population consuming water with negligible fluoride concentration. *Community Dentistry and Oral Epidemiology,* **26**(6), pp. 382-393.

ZERO, D., FU, J., ESPELAND, M. and FEATHERSTONE, J., 1988. Comparison of fluoride concentrations in unstimulated whole saliva following the use of a fluoride dentifrice and a fluoride rinse. *Journal of Dental Research,* **67**(10), pp. 1257-1262.

ZHANG, W., JOHNSON, T.J., SELTZER, T. and BICHARD, S.L., 2010. The revolution will be networked: The influence of social networking sites on political attitudes and behavior. *Social Science Computer Review,* **28**(1), pp. 75-92.

ZHANG, W., LIU, Y., SHI, X., JING, X., CHEN, J., LI, C., CUI, Z. and MA, J., 2017. Public Knowledge and Attitude Toward Organ Donation in Tianjin, China. *Transplantation,* **101**, pp. S68-S69.

ZIPKIN, I., LIKINS, R.C., MCCLURE, F.J. and STEERE, A.C., 1956. Urinary fluoride levels associated with use of fluoridated waters. *Public Health Reports,* **71**(8), pp. 767-772.

ZOBER, A., MALLINCKRODT, M.G.V. and SCHALLER, K., 1977. Renal fluoride excretion as a useful parameter for monitoring hydrofluoric acid-exposed persons. *International Archives of Occupational and Environmental Health,* **40**(1), pp. 13-24.

ZOHOORI, F.V., DUCKWORTH, R.M., OMID, N., O'HARE, W.T. and MAGUIRE, A., 2012. Fluoridated toothpaste: usage and ingestion of fluoride by 4-to 6-yr-old children in England. *European Journal of Oral Sciences,* **120**(5), pp. 415-421.

ZOHOORI, F.V., BUZALAF, M., CARDOSO, C., OLYMPIO, K., LEVY, F., GRIZZO, L., MANGUEIRA, D., SAMPAIO, F. and MAGUIRE, A., 2013. Total fluoride intake and excretion in children up to 4 years of age living in fluoridated and non-fluoridated areas. *European Journal of Oral Sciences,* **121**(5), pp. 457-464.

ZOHOORI, F.V., WALLS, R., TEASDALE, L., LANDES, D., STEEN, I., MOYNIHAN, P., OMID, N. and MAGUIRE, A., 2013. Fractional urinary fluoride excretion of 6–7-year-old children attending schools in low-fluoride and naturally fluoridated areas in the UK. *British Journal of Nutrition,* **109**(10), pp. 1903-1909.

ZOHOORI, F.V., WHALEY, G., MOYNIHAN, P. and MAGUIRE, A., 2014. Fluoride intake of infants living in non-fluoridated and fluoridated areas. *British Dental Journal,* **216**(2), pp. E3.

ZOHOURI, F.V., MAGUIRE, A. and MOYNIHAN, P.J., 2006. Sources of Dietary Fluoride Intake in 6–7-Year-Old English Children Receiving Optimally, Sub-optimally, and Non-fluoridated water. *Journal of Public Health Dentistry,* **66**(4), pp. 227-234.

ZOHOURI, F. and RUGG-GUNN, A., 2000. Total fluoride intake and urinary excretion in 4–year–old Iranian children residing in low-fluoride areas. *British Journal of Nutrition,* **83**(01), pp. 15-25.

ZUIDGEEST, M., HENDRIKS, M., KOOPMAN, L., SPREEUWENBERG, P. and RADEMAKERS, J., 2011. A comparison of a postal survey and mixed-mode survey using a questionnaire on patients' experiences with breast care. *Journal of Medical Internet Research*, *13*(3).

APPENDIX 1

Teesside University
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Teesside University

PRIVATE AND CONFIDENTIAL

Direct Line: 01642 342750

21st May 2015

Vida Zohoori
School of Health & Social Care
Teesside University

Dear Vida

Study No 065/15 - Assessment of contemporary and recent biological markers of human exposure to fluoride. Researcher: Idowu Oladipo Samson. Supervisor: Vida Zohoori.

Decision: Approved with Conditions

Thank you for submitting an amended application pack. The application was presented on a TU Request for Ethical Approval form.

I have reviewed and approved your application on 21st May 2015 and your study may proceed as it was described in your application pack, **on the condition that the comments detailed below are addressed:**

Section 7 and throughout:
Amend the recruitment strategy for Study II so that people are given the PIS and have time to consider it and have to contact the researchers if they want to ask questions, or take part. It is not acceptable to visit the home of anyone who has not already had time to consider the PIS and has already stated that they wish to be visited by a researcher.

Thank you for your response. Please ensure that the researcher will only phone, or make *any* other contact with those people who have *already* returned a completed Reply Slip giving their consent to be contacted.

With respect to University Staff participants it is clear, in your response, that you will follow that sequence - but - due to the phrasing, it is ambiguous with respect to those contacted via Schools.

Section 7:
Please verify you will obtain approval from the relevant Assistant Deans of Research prior to attempting to recruit from other Schools. Clause 1.4.1 of the University Policy, Procedures and Guidance Notes on Research Ethics, stipulates this is required

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Please ensure that you obtain approval from the relevant Assistant Deans of Research prior to attempting to recruit from other *TU* Schools. You can recruit from the School of H&SC without such specific permission but you cannot recruit from any other TU School without the relevant AD(R)s permission. Please refer to the University Policy cited above if you remain in any doubt about what is required for recruitment in TU.

Please note:

If another body was not named as the Sponsor, in the application documents reviewed, Teesside University, acting through its School of Health & Social Care, will act as Sponsor for the project.

Where applicable, your study may only proceed when you have also received written approval from any other ethical committee (e.g. NRES) and operational / management structures relevant (e.g. Local NHS R&D). A copy of this approval letter **must** be attached to applications to any other ethical committee. If applicable please forward to me a copy of the approval letter from NRES before proceeding with the study.

In all cases, should you wish to make any substantial amendment to the protocol detailed, or supporting documentation included, in your approved application pack (other than those required as urgent safety measures) you must obtain written approval for those, from myself and all other relevant bodies, prior to implementing any amendment. Details of any changes made as urgent safety measures must be provided in writing to myself and all other relevant bodies as soon as possible after the relevant event; the study should not continue until written approval for those changes has been obtained from myself and all other relevant bodies.
On behalf of the School of Health & Social Care Research Governance and Ethics Committee please accept my best wishes for success in completing your study.

Yours sincerely

Dr. Alasdair MacSween
Chair
Research Governance and Ethics Committee
School of Health & Social Care

APPENDIX 2

Teesside University
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Teesside University

PRIVATE AND CONFIDENTIAL

Direct Line: 01642 384124

6th October 2015

Vida Zohoori
School of Health & Social Care
Teesside University

Dear Vida

Study No 119/15 - Assessment of contemporary and recent biological markers of human exposure to fluoride in Nigeria. Researcher: Idowu Oladipo Samson. Supervisor: Vida Zohoori.

Decision: Approved

Thank you for submitting an amended application pack. I am pleased to confirm that the comments raised by the School of Health & Social Care Research Governance and Ethics Committee have been addressed in your amended application pack and your study has been approved through Chair's Action. Your study may proceed as it was described in your approved application pack. The application was presented on a TU Request for Ethical Approval form.

Please note:

If another body was not named as the Sponsor, in the application documents reviewed, Teesside University, acting through its School of Health & Social Care, will act as Sponsor for the project.

Where applicable, your study may only proceed when you have also received written approval from any other ethical committee (e.g. NRES) and operational / management structures relevant (e.g. Local NHS R&D). A copy of this approval letter **must** be attached to applications to any other ethical committee. If applicable please forward to me a copy of the approval letter from NRES before proceeding with the study.

In all cases, should you wish to make any substantial amendment to the protocol detailed, or supporting documentation included, in your approved application pack (other than those required as urgent safety measures) you must obtain written approval for those, from myself and all other relevant bodies, prior to implementing any amendment. Details of any changes made as urgent safety measures must be provided in writing to myself and all other relevant bodies as soon as possible after the relevant event; the study should not continue until written approval for those changes has been obtained from myself and all other relevant bodies.

On behalf of the School of Health & Social Care Research Governance and Ethics Committee please accept my best wishes for success in completing your study.

Yours sincerely

Dr. Alasdair MacSween
Chair
Research Governance and Ethics Committee
School of Health & Social Care

APPENDIX 3

Dr Vida Zohoori
School of Health and Social Care
Constantine Building
Teesside University
Middlesbrough, TS1 3BA, UK

Faculty of Medical Sciences
Newcastle University
The Medical School
Framlington Place
Newcastle upon Tyne
NE2 4HH United Kingdom

FACULTY OF MEDICAL SCIENCES: ETHICS COMMITTEE

Dear Vida,

Title: Assessment of contemporary and recent biological markers of human exposure to fluoride
Application No: 00878 2015
Start date to end date: 2015 to 2016

On behalf of the Faculty of Medical Sciences Ethics Committee, I am writing to confirm that the ethical aspects of your proposal have been considered and your study has been given ethical approval.

The approval is limited to this project: **00878/2015**. If you wish for a further approval to extend this project, please submit a re-application to the FMS Ethics Committee and this will be considered.

During the course of your research project you may find it necessary to revise your protocol. Substantial changes in methodology, or changes that impact on the interface between the researcher and the participants must be considered by the FMS Ethics Committee, prior to implementation.*

At the close of your research project, please report any adverse events that have occurred and the actions that were taken to the FMS Ethics Committee.*

Best wishes,
Yours sincerely
K. Sutherland
Kimberley Sutherland
On behalf of Faculty Ethics Committee

cc.
Professor Daniel Nettle, Chair of FMS Ethics Committee
Ms Lois Neal, Assistant Registrar (Research Strategy)

*Please refer to the latest guidance available on the internal Newcastle web-site.

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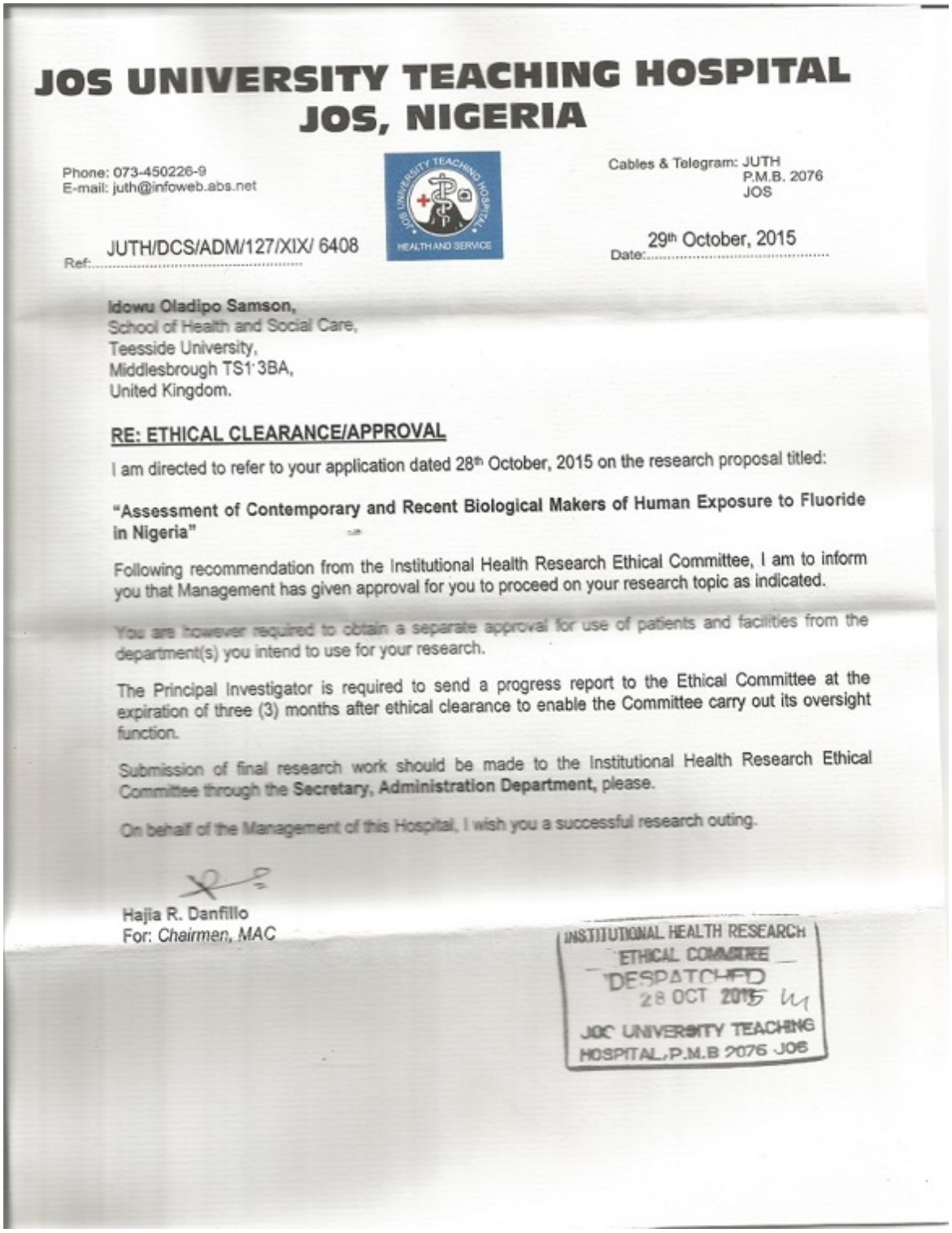
APPENDIX 4



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Ref: JUTH/DCS/ADM/127/XIX/ 6408

Date: 29th October, 2015

Idowu Oladipo Samson,
School of Health and Social Care,
Teesside University,
Middlesbrough TS1 3BA,
United Kingdom.

RE: ETHICAL CLEARANCE/APPROVAL

I am directed to refer to your application dated 28th October, 2015 on the research proposal titled:

"Assessment of Contemporary and Recent Biological Makers of Human Exposure to Fluoride in Nigeria"

Following recommendation from the Institutional Health Research Ethical Committee, I am to inform you that Management has given approval for you to proceed on your research topic as indicated.

You are however required to obtain a separate approval for use of patients and facilities from the department(s) you intend to use for your research.

The Principal Investigator is required to send a progress report to the Ethical Committee at the expiration of three (3) months after ethical clearance to enable the Committee carry out its oversight function.

Submission of final research work should be made to the Institutional Health Research Ethical Committee through the **Secretary, Administration Department,** please.

On behalf of the Management of this Hospital, I wish you a successful research outing.

Hajia R. Danfillo
For: *Chairmen, MAC*

INSTITUTIONAL HEALTH RESEARCH ETHICAL COMMITTEE
DESPATCHED
28 OCT 2015
JOS UNIVERSITY TEACHING HOSPITAL, P.M.B 2076 JOS

APPENDIX 5

Teesside University
Middlesbrough Tees Valley
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17th February, 2016

Dear ----------,

SURVEY ON ACCEPTABILITY OF BIOLOGICAL MARKERS FOR MONITORING OF HUMAN EXPOSURE TO FLUORIDE

We write to request your assistance in facilitating some research we hope to carry out in Middlesbrough schools. We hope to gather information on what people think about providing samples of urine, saliva, blood, hair and nails for research purposes. Parents of 4-5 year old in randomly selected schools throughout Middlesbrough will be asked to participate in this survey. Their responses will help us to identify which tissues or fluid is acceptable for future monitoring of fluoride exposure in children. Your school has been selected randomly to participate.

This study is being carried out by Mr Idowu Oladipo Samson, a PhD student at Teesside University, and has ethical approval from The School of Health and Social Care Research Ethics Committee (a copy of the approval is enclosed). He is conducting research on assessment of human exposure to fluoride using human biological tissues/fluids under the supervision of Professor Vida Zohoori and Dr Ralph Duckworth (School of Health and Social Care, Teesside University) as well as Dr Ruth Valentine (Dental School, Newcastle University).

Fluoride is mainly in the body with calcified tissues such as bones and teeth. It is found naturally in foods that we eat, water we drink and toothpaste we use. Fluoride has been proven to be effective in the control of tooth decay but excessive intake of fluoride may lead to discoloration or mottling of teeth. Monitoring of exposure to fluoride is therefore very important, as recommended by the World Health Organization (WHO).

The questionnaire will be completed anonymously. All information collected will be treated in strictest confidence. Neither the school nor individual children/parents will be identifiable in any reports that are written. It will only take 15 minutes to complete the questionnaire. A copy of the questionnaire included for your reference.

As a token of our thanks we would provide a school with £1 voucher for each returned questionnaire. This voucher would be considered as income and so it may affect your School's tax liability and/or your entitlement to any income dependant grants or payments your School receives. As we don't know your circumstances, we have not deducted any UK income tax, nor National Insurance for you and it's up to you to let the appropriate agencies know if this is relevant to your circumstances.

Once we have received your consent to approach children to participate in the study, Mr Idowu will give the invitation pack containing the questionnaire and parent's invitation to the Head teacher, who will pass it to the children within the age group for the study. The researcher, Mr Idowu will be at the school to collect the completed questionnaire.

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Based on the information obtained from the questionnaire, ONLY parents who are happy to provide samples will be contacted by the researcher for further studies.

We will contact you over the next few days to know if you are interested in your school taking part. If so, Mr Idowu would then come and talk to you more about the study and answer any questions you might have.

In the meantime, if you have any questions regarding this study or research projects in general, please contact Mr Idowu Oladipo on 01642384157 or by email at o.idowu@tees.ac.uk or supervisor Prof Vida Zohoori on 01642342973 or email v.zohoori@tees.ac.uk.

Yours Faithfully,

V Zohoori

Professor Vida Zohoori

Research team:
Researcher, Mr. Idowu Oladipo Samson, Teesside University, UK;
Director of studies: Prof Vida Zohoori, Teesside University, UK;
Co-supervisors: Dr Ruth Valentine, Newcastle University, UK; and Dr Ralph Duckworth, Newcastle University, UK

APPENDIX 6

Teesside University
Middlesbrough Tees Valley
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Teesside University

6th Nov 2015

Dear Parent/Guardian

LETTER OIF INVITATION FOR RESEARCH PARTICIPATION

We would like to invite you and/or your child to take part in a research study by the School of Health and Social Care, Teesside University, UK.

We would like to find out what people think about providing samples of urine, saliva, hair and nails for medical research.

For the purpose of this research study, you will be asked to complete a questionnaire. There is also a brief information sheet and reply slip attached to the study pack for you to complete if you are happy to participate in future studies which would involve providing any among (a) 24-hour urine to enable us measure your and/or your child's fluoride excretion and (b) saliva, blood, hair and nails in order to measure the amount of fluoride that is stored in the body.

If you are happy to complete the questionnaire, the researcher Mr Idowu will pick it up in your child's school on an arranged date. We hope you will consider taking part and look forward to hearing from you.

Yours faithfully,
V zohoori
Professor Vida Zohoori
School of Health and Social Care, Teesside University

APPENDIX 7

Teesside University
Middlesbrough Tees Valley
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Teesside University

24th April, 2016

Dear Parent/guardian

We would like to invite you and/or your child to take part in our research study by the school of Health and Social Care, Teesside University, UK.

We are carrying out a study to find out the amount of fluoride that is stored in urine, blood, saliva, hair and nail. Fluoride is found naturally in the water we drink, food we eat and toothpaste we use. People of this community are been asked to take part in this study because fluoride helps to prevent tooth decay. Whatever we learn about our community will be compared with information from other communities and will help in establishing a programme to prevent tooth decay in the population living in the community.

The study is simple. It involves collecting few samples of you and your child's urine, blood, saliva, hair, nail and a sample of your drinking water. Plastic containers will be provided by the people in charge of the study. Please let us know whether or not you and your child would be interested in getting more details and possibly that you and your child takes part in this study.

We will kindly ask you to complete the form attached. You may contact the office of the head teacher of the school if you have any questions you want answered first. We will be available at your child's school on an appointed date to answer any question you might have. You can also call the research mobile number 08180458893 for any enquiry.

We look forward to hearing from you and we sincerely hope you take part in the study.

Yours faithfully,

Idowu Oladipo

School of Health and Social Care,

Teesside University, UK

08180458893

APPENDIX 8

Teesside University
Middlesbrough Tees Valley
TS1 3BA UK
www.tees.ac.uk

Teesside University

24th April, 2016

Dear Sir,

Assessment of Contemporary and Recent biological markers of human exposure to Fluoride

Researcher: Mr Idowu Oladipo Samson

We humbly request your assistance in facilitating some research we hope to carry out in some schools within Plateau State, Nigeria. We hope to gather information on the most reliable human biological tissue/fluid for monitoring short term and long term exposure to fluoride.

My name is Idowu Oladipo Samson, and I am a PhD student at Teesside University, Middlesbrough, UK. I am conducting research on assessment of human exposure to fluoride using human biological tissues/fluids under the supervision of Dr Vida Zohoori, School of Health and Social Care, Teesside University; Dr Ruth Valentine, Dental School, Newcastle University, UK and Dr Ralph Duckworth, Dental School, Newcastle University, UK. The School of Health and Social Care, Teesside University has given approval to approach schools in Plateau State, Nigeria for my research. University of Jos Teaching hospital Ethics Committee has also approved the study. A copy of their approval is contained with this letter. I invite you to consider taking part in this study. This study will meet the requirements of the Research Ethics Committee (Human) of the Teesside University, Middlesbrough UK and Ethics Committee of University of Jos Teaching Hospital.

Fluoride is mainly in the body with calcified tissues such as bone and teeth. It is found naturally in foods that we eat; water we drink and toothpaste we use. Fluoride has been proven to be effective in the control of dental caries also known as tooth decay but excessive intake of fluoride may lead to discoloration or mottling of teeth. Monitoring of exposure to fluoride is therefore very important as recommended by World Health Organization (WHO). Dental fluorosis is known to occur in some part of Nigeria with high percentage in Plateau State and this has been attributed to the amount of fluoride in drinking water, but other factors that might be responsible for its occurrence have not been documented. Monitoring of fluoride in human biological tissues and fluids have proven to be very effective in assessment of the amount of fluoride that is retained in the body over short and long period of time. This study will find out which among 24-hour urine, blood, saliva, nail and hair will be most effective for assessment of short term and long term exposure. In addition we hope to find out how differences in genetic arrangement of the individual affects the way they handle fluoride in the body.

With your permission we would like to recruit all 4-5 year old children and their parents aged 20-60 years. Parents would be asked to complete a Food Frequency Questionnaire for themselves and child. Parents will also be asked to collect their child's 24-hour urine to enable us measure their fluoride excretion and as well saliva, hair, nail in order to measure the amount of fluoride that is

Teesside University
Middlesbrough Tees Valley
TS1 3BA UK
www.tees.ac.uk

retained in the body. We will arrange with the parents when a laboratory technician will collect blood samples. The other samples will be provided on thesame day and stored in the freezer for analysis. DNA samples will be extracted from the blood sample and sent to the UK for analyses and results will be kept in Newcastle University, UK and no result will be fed back to participants. Permission will be sought from the child and their parents prior to their participation in the research. Only those whose parents consent will participate.

All information collected will be treated in strictest confidence. Neither the school nor individual children/parents will be identifiable in any reports that are written. Participants may withdraw from the study at any time without penalty. The role of the school is voluntary and the School Principal may decide to withdraw the school's participation at any time without penalty.

For further clarification, we enclosed a study pack containing parent information booklet, Food Frequency Questionnaire, instruction for collection of samples, urine information sheet, Parent informed consent which will be given to parents of all 4-5 old before they decide if they would like to take part in the study. Once we have received your consent to approach children to participate in the study, we will give the invitation pack to the head teacher who will pass it to the children within the age group for the study. We, will

- be available in the school to answer questions the parent might have on an arranged date.
- arrange for informed consent to be obtained from participants' parents and assent from the child
- Arrange a time with the participants for data collection to take place in their home.

An introductory session will be organised in your school where there will be further clarification about the study.

We will contact you to know if you are interested in taking part. If you are willing to take part, Idowu will return to answer any questions you might have before you decide whether or not to take part.

Thank you

Yours Faithfully,

Mr. Idowu Oladipo
PhD Student, School of Health and Social Care,
Teesside University, UK
08180458893

APPENDIX 9

ABBREVIATED MEDICAL HISTORY

Has your child ever suffered from any of the following conditions?

	Yes	No		Yes	No
Asthma			Cancer		
Tuberculosis			HIV/AIDS		
Diabetes Mellitus			Venereal disease		
Heart disease			Leukemia		
Malaria			Hemophilia		
Urinary infection			Hepatitis		
Oral diseases			Measles		
Thyroid disease			Rubella		
Kidney disease			Ebola		
Respiratory Tract Infections			Chronic Obstructive Pulmonary Diseases (COPD)		
Other disease (if yes please list below)					

Please if any of the above is Yes, Have you been treated or currently receiving treatment……

……

Operation/surgical ……

Allergic to ……..

Do you have any dietary restrictions ……...

Are you currently undergoing any medical/dental treatment (Yes/No) ……

Have you been using any medication (use of fluoride gels) Yes/No. if yes, please list below

……

……

APPENDIX 10

CONSENT FORM (*adult participants*)
Name of the project: The Assessment Level of Fluoride Exposure: using 24-h urine, saliva, blood, hair and nails (ALFE)

Research team: Researcher, Mr. Idowu Oladipo Samson, Teesside University; Director of studies, Dr Vida Zohoori, Teesside University; Supervisor, Prof Tim Thompson, Teesside University; Supervisor, Dr Ruth Valentine, Newcastle University; Supervisor, Dr Ralph Duckworth, Newcastle University.

Name: _____ **Male/Female**

Age: _____ **(years)**

All information collected and used for the purpose of this study will remain anonymous and confidential. The information will be collected and stored on paper and on computer. The paper documents will be stored in a locked filling cabinet and the research team will be the only people with access. The computer documents will be stored on a password protected computer in password protected files. All information will be stored at the University of Teesside.

Please read the following questions carefully. If you answer yes to the question, please put your initial in the box (on the same line as the question). Surely, if the answer is 'NO', then the 'NO' box should be initialed. Please repeat this for each of the question asked.

	Please Initial box
1. I have read and understood the information sheet for the above study and had time to think about it.	☐
2. I understand that my participation in this study is entirely voluntary.	☐
3. I understand that I can withdraw my consent at any stage without giving reason and without prejudice.	☐
4. I understand that all information will be treated as confidential, and that I will not be identified in any way.	☐
5. I understand that my signing and returning this form, I am giving my consent to participate in this study.	☐
6. I verify that I am free of any of the exclusion criteria and meets the inclusion criteria	☐

If you have answered yes to all the above questions, please complete the details below:

Signature: …….. Date: ……..

I can confirm that I have explained to the participant the nature of this study and have given adequate time to answer any question concerning it.

Signed: …….. Date: ……..

If you have any questions then you can ask Mr. Idowu Oladipo Samson on Research Mobile number: 07467915304 or 01642384157, email o.idowu@tees.ac.uk, School of Health and Social Care, Teesside University

Thank you for agreeing to take part in this study. When completed: 1 copy to participant, 1 copy to researcher file

APPENDIX 11

CONSENT FORM

Title of Project: Storage of anonymised DNA samples

Name of Researchers: **Mr. Idowu Oladipo Samson, Teesside University, UK; Director of studies, Dr Vida Zohoori, Teesside University, UK; Supervisor, Dr Ruth Valentine, Newcastle University, UK; Supervisor, Dr Ralph Duckworth, Newcastle University, UK.**

Please initial box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to take part in the above study. ☐

Name of Patient	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 for patient; 1 for researcher

APPENDIX 12

CONSENT FORM (*adult participants*)
Name of the project: The Assessment of Contemporary and Recent Biological Markers of Human Exposure to Fluoride

Research team: Researcher, Mr. Idowu Oladipo Samson, Teesside University, UK; Director of studies, Dr Vida Zohoori, Teesside University, UK; Supervisor, Dr Ruth Valentine, Newcastle University, UK; Supervisor, Dr Ralph Duckworth, Newcastle University, UK.

Name: _____

Please read the following statements carefully. If you agree, please put your initial in the box (on the same line as the statement).
Please Initial box

1. I have read and understood the information sheet for the above study and had time to think about it. ☐
2. I understand that my participation in this study is entirely voluntary. ☐
3. I understand that I can withdraw my consent at anytime prior to commencement of sample analysis without giving a reason and without prejudice. ☐
4. I understand that all information will be treated as confidential, and that I will not be identified in any way. ☐
5. I understand that by signing and returning this form, I am giving my consent to participate in this study. ☐
6. I agree that my child who is aged 4-5 years old to take part in this study I don't feel they are able to sign an assent form themselves so I have not asked them to. ☐
7. I agree for my child , who is aged 4-5 years old to take part in this study (if they want to) and have asked them to complete an assent form themselves if they want to take part ☐
8. I verify that I am free of any of the exclusion criteria and meet the inclusion criteria ☐
9. I understand that my DNA or my child's DNA (aged 4-5) will be taken to the UK to investigate genes that are affected by fluoride in the body. ☐
10. I understand that my DNA extract and my child's will be held in the UK indefinitely ☐

If you have initialed the above statements, please complete the details below:
Name: ..... (Block Letters)
Signature: ..... Date: .....
I can confirm that I have explained to the participant the nature of this study and have given adequate time to answer any question concerning it.
Name: ..... (Block Letters)
Signed: ..... Date: .....

If you have any questions then you can ask Mr. Idowu Oladipo Samson on Nigeria Research Mobile number: 08180458893.
Thank you for agreeing to take part in this study. When completed: 1 copy to participant, 1 copy to researcher file

APPENDIX 13

Subject ID:

FOOD FREQUENCY QUESTIONNAIRE

ASSESSMENT OF CONTEMPORARY AND RECENT BIOLOGICAL MARKERS OF EXPOSURE TO FLUORIDE



INSTRUCTION

Please record the food you ate over the past months 3 months. The official space should be left blank as this will be completed by the researcher on return of this questionnaire.

We don't need to know about the amount of each food, just the food you ate

Subject ID:

Table 1: Record of drinks

S/N	Drinks	Yes	No	Frequency of use/week				Official use
				Once	Twice	Daily	Occasionally	
1	Bottled water Brand (s):							
2	Tap water							
3	Tea Type (s)/Brand (s):							
4	Coffee Type (s)/Brand (s):							
5	Hot Chocolate							
6	Any other hot drink Type (s)/Brand (s):							
7	Squash and Cordials Ready to drink Type (s)/Brand (s): Prepared by adding water Type (s)/Brand (s):							
8	Non-carbonated soft drink Brand (s):							
9.	Carbonated soft drink Brand (s):							

Subject ID:

Table 2: Food record

S/N	Food	Yes	No	Frequency of use/week				Method of preparation	Official use
				Once	Twice	Daily	Occasionally		
10	Fruits Raw Types (s): Cooked Ready to eat: Home-made:								
11	Vegetables Raw Type (s): Cooked Type (s):								
12	Soup and Gravy Homemade Ready to eat								
13	Bread Ready to eat Type (s): Homemade								

Subject ID:

S/N	Food	Yes	No	Frequency of use/week				Method of preparation	Official use
				Once	Twice	Daily	Occasionally		
14	Rice and pasta Home made Ready to eat Brand (s):								
15	Fish and sea food Fried Canned Brand (s):								
16	Breakfast cereals Type (s)/Brand (s):								
17	Meat and meat products Ready to eat Homemade								
18	Confectionaries, cakes and sweets Type (s)/Brand (s)								
19	**Others**								

Subject ID:

S/N	Food	Yes	No	Frequency of use/week				Method of preparation	Official use
				Once	Twice	Daily	Occasionally		

APPENDIX 14

Food Frequency Questionnaire (FFQ)

THE ASSESSMENT OF CONTEMPORARY AND RECENT BIOLOGICAL MARKER OF HUMAN EXPOSURE TO FLUORIDE

(*This questionnaire asks about your food and drink intake over the last year and it is to be completed either by*

1. ***The person taking part in the study alone***
2. ***The person taking part in the study with the assistance of a family member***
3. ***The person taking part in this study with the assistance of the researcher.***
4. ***The researcher during an interview***

Participant ID number_____

Location _____

Date _____

If you have any questions about this form or how it should be completed, please do not hesitate to contact the researcher on Nigeria research mobile number with 08180458893.

Question about water sources at home

1. Where do you get water for drinking at home?

a. Public water supply (tap) b. Well c. Borehole d. sachet water

2. Where do you get water for cooking at home?

a. Public water supply (tap) b. Well c. Borehole d. sachet water

Question about water sources at school/work

b. Where do you get water for drinking at school?

a. Public water supply (tap) b. Well c. Borehole d. sachet water

S/N	Food eaten	No of times			No of Days/Week							Amount/ day on serving	Official use
		/Month	/Fortnight	Never	1	2	3	4	5	6	7		
A	**WATER**												
1	Tap water												
2	Well water												
3	Borehole water												
4	Sachet water												
5	Bottled water												
6	Natural water (river, stream, rain)												
B	**BEVERAGES**												
7	Tea bags with milk Brand: Type of water:												
8	Herbal tea bags Type of water:												
9	Coffee Brand/Type: Type of water:												
10	Chocolate drink Type of water:												
11	Powdered milk (sachet or tin) Type of water:												
12	Alcoholic drink e.g. wine, beer Type/Brand:												
13	Sugar (in tea and coffee or pap) Type/Brand:												
14	Soft drink e.g. coke. Brand:												
15	Fruit drink e.g. apple Brand:												
C	**DIARY PRODUCTS**												
17	Egg (fried/Boiled)												
18	Cheese (fried/Boiled)												
19	Processed milk Type/brand:												
20	Other milk e.g. human, goat, cow etc												
21	Butter Type/brand:												
C	CEREALS												

S/N	Food eaten	No of times			No of Days/Week							Amount/ day on serving	Official use
		/Month	/Fortnight	Never	1	2	3	4	5	6	7		
A	**WATER**												
22	Rice (Boiled/fried/jollof)												
23	Wheat												
24	Maize (boiled/Roasted/mixed with other food)												
25	Maize (processed)Pap (Akamu)/kunu												
25	Sorghum: pap (brown) akamu												
26	Paster e.g. Spaghetti (Boiled/Jollof)/ Macarroni Brand: Indomine noodles Brand (s):												
27	Bread Brand/Type:												
28	Breakfast cereals e.g. cornflakes, branflakes												
D	**ROOT AND TUBERS**												
	Yam (boiled/roasted/fried /processed)												
	Potato (boiled/fried/roasted /mixed with other food) Type:												
	Cassava (Processed) Type:												
	Plantain (Boiled/fried/roasted)												
	POULTRY/MEAT AND FISH												
	Meat Type:												
	Salted/dried meat Type												

S/N	Food eaten	No of times			No of Days/Week							Amount/ day on serving	Official use
		/Month	/Fortnight	Never	1	2	3	4	5	6	7		
A	**WATER**												
	Fish (boiled/fried/smoked/salted dried)												
	Canned fish (tuna, sardine)												
	Shell fish (shrimp, crab)												
	Snail												
	Game meat Type (s):												
	Meat products e.g. meat pie, gala etc.												
	BEANS												
	Beans (cooked fresh/ mixed with other food) Type:												
	Processed beans Type:												
	Vegetable Type:												
	Fruits **Type:**												
	Others (list below)												

Thank you for completing the FFQ

APPENDIX15

USEOF DENTRIFICE AND FLUORIDE SUPPLEMENT BY CHILDREN/ADULT

Subject ID

Date: _____

Personal details

Sex: Male ☐ Female ☐

Date of birth (Month/Year): _____/ _____

Home address: _____

_____

Name of child's school: _____

_____

Anthropometric data

Weight: _____kg

Height: _____cm

Occupation of the father or responsible family member

Manual Technical Professional

Level of education of mother

None Elementary Secondary College Professional

Tooth brushing Habit

Do (you/child) use toothbrush? Yes No

Do (you/child) use toothpaste? Yes No

The start age for the use of toothpaste (Year and month): _____

The type of regularly used toothpaste (Brand and name): _____

The number of times you brush (Per day): _____

Only for ages 4 – 5 years

The person who brushes the child's teeth: _____

The person who puts toothpaste on the toothbrush: _____

Do you usually buy the same toothpaste for adult and children in the family? Yes No

Smear Pea-size Regular

A B C

Has (child/parent) ever taken medicine with fluoride for preventing dental caries such as drops, tablets or vitamins?

Fluoride drops Fluoride tablets Vitamins with fluoride

Are (you/child) taking any of these at the present time? Yes No

At what age did (you/child) start taking these medicines? _____

Please tell us the brand (you/child) has used

Drops_____

Tablets_____

Vitamins_____

Who prescribes the use of the medicine? Dentist Doctor Others

How many times a day do (you/child) take these supplements?

Once Twice Three times More than three times

APPENDIX 16

QUESTIONNAIRE

THE USE OF HUMAN TISSUE AND FLUID FOR RESEARCH/MEDICAL PURPOSE: HOW ACCEPTABLE WOULD YOU FIND IT

Teesside University is sponsoring this project for the purposes of research governance

(PLEASE COMPLETE THIS QUESTIONNAIRE ON BEHALF OF YOUR 4-5 YEAR OLD CHILD *AND RETURN YOUR COMPLETED QUESTIONNAIRE IN THE ENCLOSED ENVELOPE TO YOUR CHILD'S SCHOOL.)*

School of Health and Social Care,
Constantine Building, Teesside
University, TS1 3BA,
Middlesbrough

QUESTIONNAIRE

(Please complete this questionnaire on behalf of your 4-5 year old child)

Hello, we are researchers from Teesside University. We are carrying out a study to find out what parents think about having to collect their child's urine, saliva, blood, hair and nail samples to be tested for medical research. To help our work we would like to receive your response to the following questions.

PART 1

1. What is your child's sex? Male/Female
2. What is your child's year of birth?

   _____

3. Please tick your ethnic background.

Black or Black British-Caribbean		White Caucasian or White British	
Black or Black British-African		Chinese	
Black other		Mixed White and Asian/Black African/Caribbean	
Asian or Asian British		Mixed other	
Asian other		Not Known	

4. Do you have a job? YES/NO
5. Please tick the last grade you completed in school or the grade you are currently studying.

GCSEs	O-Levels	A-Levels	Diploma	Degree	Vocation	Others	None

6. If you have a degree can you tell us what subject your degree course is/was?

   _____

7. What is your religion? Please Circle

No religion	Muslim	Christian	Buddhist	Hindu	Jewish	Sikh	Prefer not to say

PART 2

Now we would like to ask you some questions about how you would feel if your child is asked to provide any samples (urine, saliva, blood, hair, and nail) for medical research. We just want to know what you think about the way the samples are collected: Are they ok? Could you do it? Would it be easy for you to do?

Collecting your child's urine for 24 hours: *For a 24-hour urine sample you would be told to collect your child's urine every time he/she goes to the toilet over 24 hours using the containers that we would give you (storage container, disposable cups and funnel). You would then seal it and the researcher would visit to collect the sample for analysis.*

8. What do you feel about having to collect your child's urine for 24 hours? Please Circle

Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable

9. Now that you know what you would have to do to collect your child's urine for 24 hours, can you answer on a scale of 1-10 by selecting a number how you would find collection of urine over 24 hour. 1 means very hard and 10 means very easy

1	2	3	4	5	6	7	8	9	10

Collecting your child's urine at set times (spot sample): *For a spot sample, your child would urinate at set times during one to two days and you would put a small amount of each in the container you would be provided with. If you chose to do this, before you started you would be told how many days you needed to do this for depending on the particular test being done.*

10. What do you feel about having to collect spot urine samples?

Please tick the most appropriate for each day

	Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable
One day						
Two days						

11. Now that you know what you would have to do to collect your child's spot urine samples, can you answer on a scale of 1-10 by selecting a number on the scale how you would find it. 1 means very hard and 10 means very easy

1	2	3	4	5	6	7	8	9	10

Collecting your child's saliva: *To get your child's saliva, first he/she would swallow the saliva in his/her mouth, and then as the mouth starts to fill up again, he/she would need to allow the saliva to flow into a plastic bottle as shown for 2-3 minutes.*

12. What do you feel about the **saliva** collection? Please Circle

Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable

13. Now that you know what you would have to do to collect your child's saliva, can you answer on a scale of 1-10 by selecting a number on the scale how you would find it. 1 means very hard and 10 means very easy

1	2	3	4	5	6	7	8	9	10

Blood tests

A trained nurse would take the blood sample. The nurse would clean your child's skin with alcohol and collect a small amount of blood (2 tea spoons) as shown. This procedure is identical to that used at the doctor's surgery and in hospital.

How do you feel about the way your child's blood would be taken? Please Circle

Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable

14 Now that you know what taking your child's blood would mean, can you answer on a scale of 1-10 by ticking the box on the scale how you would find it? 1 means very hard and 10 means very easy.

1	2	3	4	5	6	7	8	9	10

Collecting nail clippings: *Before clipping, you would need to wash your child's hands and feet with water and soap. Then clip his/her fingernails and toenails using a clip or cut with scissors and store them in separate labelled sachets that we would provide.*

15 What do you think about being asked to collect your child's nails? Please Circle

Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable

16 Now that you know what you would have to do to collect your child's nails, can you answer on a scale of 1-10 ticking the box on the scale how you would find it. 1 means very hard and 10 means very easy.

1	2	3	4	5	6	7	8	9	10

Collecting hair: *Before collecting your child's hair, you would need to wash and dry the hair. You would be asked to cut about 1 inch of your child's hair as shown in the diagram. This would need to be from the back of the head close to the scalp. Then you would put it in a sachet provided.*

17 What do you think about being asked to collect your child's hair? Please Circle

Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable

18 Now that you know what you would have to do to collect your child's hair, can you answer on a scale of 1-10 by ticking the box on the scale how you would find it. 1 means very hard and 10 means very easy

1	2	3	4	5	6	7	8	9	10

19 If you were asked to provide any of the above types of sample from your child, which would you be willing to provide? Please tick

	24 hrs Urine	Spot Urine	Saliva	Blood	Nail	Hair
Yes						
No						

20 Please rank in order *of 1-6 by selecting a number how you prefer it. 1 means least preferred and 6 means most preferred (Please tick)*

	1	2	3	4	5	6
24 hrs Urine						
Spot Urine						
Saliva						
Blood						
Nail						
Hair						

If you would like to say anything else about the collection procedures please write it below

.....

.....

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE
Please return it to your child's school

6

AN OPPORTUNITY TO BE INVOLVED IN RESEARCH

The researchers are also looking for children aged 4-5years old who would be willing to take part in a study and provide any of the following samples – 24 hour urine, spot urine test, saliva, blood, hair and nail - for us to see how much fluoride they are exposed to. Fluoride in human body helps to strengthen the bone and it is known to prevent tooth decay. Fluoride is found naturally in foods that we consume, water we drink and most of the toothpaste we use. Within the body it is found mainly in bones and teeth. Normally the amount of this fluoride is small but high levels will lead to discolouring of teeth. It is therefore very important to know the level of fluoride in the body: if it is too high or too low. The amount in our body can be attributed to the level of fluoride in our food and other sources e.g. toothpaste, mouth rinses, or topical use from medications.

My child would like to take part? Please tick applicable box below

☐ Yes, my child would like to take part

Comments _____

_____

_____

If you are happy for your child to take part, please tick which kinds of sample you are willing to provide

24-hour urine	☐
Spot urine	☐
Saliva	☐
Blood	☐
Nails	☐
Hair	☐

If you would like your child to take part or would be willing to be contacted to discuss whether you would be willing for your child to be involved in this second part of the study, please complete the reply slip below and return it with your questionnaire to your child's school. A study pack will be sent to you containing more information about the research. If you are not interested in finding out more, please leave this section blank.

RESPONSE SLIP

Please tick as appropriate

My child and I would like to receive more information before we decide whether or not we would like to take part in the study ☐

Parent/guardian's name: .....

Child's name: .....

Parent/child's address: .....

.....

.....

Telephone: .....

Mobile: .....

Parent/guardian's signature: .....

Please return the questionnaire and reply slips to your child's school

APPENDIX 17

QUESTIONNAIRE

THE USE OF HUMAN TISSUE AND FLUID FOR RESEARCH/MEDICAL PURPOSE: HOW ACCEPTABLE WOULD YOU FIND IT

Teesside University

Teesside University is sponsoring this project for the purposes of research governance

(PLEASE COMPLETE THIS QUESTIONNAIRE FOR YOURSELF *AND RETURN YOUR COMPLETED QUESTIONNAIRE IN THE ENCLOSED ENVELOPE TO YOUR CHILD'S SCHOOL.)*

School of Health and Social Care,
Constantine Building, Teesside University, TS1 3BA,
Middlesbrough

QUESTIONNAIRE

Hello, we are researchers from Teesside University. We are carrying out a study to find out what you think about having to collect your urine, saliva, blood, hair and nail samples to be tested for medical research. To help our work we would like to receive your response to the following questions.

PART 1

1. What is your sex? Male/Female
2. What is your year of birth? _____
3. Please tick your ethnic background.

Black or Black British-Caribbean		White Caucasian or White British	
Black or Black British-African		Chinese	
Black other		Mixed White and Asian/Black African/Caribbean	
Asian or Asian British		Mixed other	
Asian other		Not Known	

4. Do you have a job? YES/NO
5. Please tick the last grade you completed in school or the grade you are currently studying.

GCSEs	O-Levels	A-Levels	Diploma	Degree	Vocation	Others	None

6. If you have a degree can you tell us what subject your degree course is/was?

   _____

7. What is your religion? Please Circle

No religion	Muslim	Christian	Buddhist	Hindu	Jewish	Sikh	Prefer not to say

PART 2

Now we would like to ask you some questions about how you would feel if you were asked to provide any sample (s) (urine, saliva, blood, hair, and nail) for medical research. We just want to know what you think about the way the samples are collected: Are they ok? Could you do it? Would it be easy for you to do?

Collecting your urine for 24 hours: *For a 24-hour urine sample you would be told to collect your urine every time you go to the toilet over 24 hours using the containers that we would give you (storage container, disposable cups and funnel). You would then seal it and the researcher would visit to collect the sample for analysis.*

8. What do you feel about having to collect your urine for 24 hours? Please Circle

Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable

9. Now that you know what you would have to do to collect your urine for 24 hours, can you answer on a scale of 1-10 by selecting a number how you would find collection of urine over 24 hour. 1 means very hard and 10 means very easy

1	2	3	4	5	6	7	8	9	10

Collecting your urine at set times (spot sample): *For a spot sample, you would urinate at set times during one to two days and you would put a small amount of each in the container you would be provided with. If you chose to do this, before you started you would be told how many days you needed to do this for depending on the particular test being done.*

What do you feel about having to collect spot urine samples?

Please tick the most appropriate for each day

	Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable
One day						
Two days						

10. Now that you know what you would have to do to collect your spot urine samples, can you answer on a scale of 1-10 by selecting a number on the scale how you would find it. 1 means very hard and 10 means very easy

1	2	3	4	5	6	7	8	9	10

Collecting your saliva: *To get your saliva, first you would swallow the saliva in your mouth, and then as the mouth starts to fill up again, you would need to allow the saliva to flow into a plastic bottle as shown for 2-3 minutes.*

11. What do you feel about the **saliva** collection? Please Circle

Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable

12. Now that you know what you would have to do to collect your saliva, can you answer on a scale of 1-10 by selecting a number on the scale how you would find it. 1 means very hard and 10 means very easy

1	2	3	4	5	6	7	8	9	10

Blood tests

A trained nurse would take the blood sample. The nurse would clean your skin with alcohol and collects a small amount of blood (2 tea spoons) as shown. This procedure is identical to that used at the doctor's surgery and in hospital.

How do you feel about the way your blood would be taken? Please Circle

Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable

14 Now that you know what taking your blood would mean, can you answer on a scale of 1-10 by ticking the box on the scale how you would find it? 1 means very hard and 10 means very easy.

1	2	3	4	5	6	7	8	9	10

Collecting nail clippings: *Before clipping, you would need to wash your hands and feet with water and soap. Then clip your fingernails and toenails using a clip or cut with scissors and store them in separate labelled sachets that we would provide.*

15 What do you think about being asked to collect your nails? Please Circle

Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable

16 Now that you know what you would have to do to collect your nails, can you answer on a scale of 1-10 ticking the box on the scale how you would find it. 1 means very hard and 10 means very easy.

1	2	3	4	5	6	7	8	9	10

Collecting hair: *Before collecting your hair, you would need to wash and dry the hair. You would be asked to cut about 1 inch of your hair as shown in the diagram.*

This would need to be from the back of the head close to the scalp. Then you would put it in a sachet provided.

17 What do you think about being asked to collect your hair? Please Circle

Totally Unacceptable	Unacceptable	Slightly unacceptable	Neutral	Slightly acceptable	Perfectly acceptable

18 Now that you know what you would have to do to collect your hair, can you answer on a scale of 1-10 by ticking the box on the scale how you would find it. 1 means very hard and 10 means very easy

1	2	3	4	5	6	7	8	9	10

19 If you were asked to provide any of the above types of sample, which would you be willing to provide? Please tick

	24 hrs Urine	Spot Urine	Saliva	Blood	Nail	Hair
Yes						
No						

20 Please rank in order *of 1-6 by selecting a number how you prefer it. 1 means least preferred and 6 means most preferred (Please tick)*

	1	2	3	4	5	6
24 hrs Urine						
Spot Urine						
Saliva						
Blood						
Nail						
Hair						

If you would like to say anything else about the collection procedures please write it below

.....

.....

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE
Please return it to your child's school

6

AN OPPORTUNITY TO BE INVOLVED IN RESEARCH

The researchers are also looking for adults aged >20 years old who would be willing to take part in a study and provide any of the following samples – 24 hour urine, spot urine test, saliva, blood, hair and nail - for us to see how much fluoride they are exposed to. Fluoride in the human body helps to strengthen the bone and it is known to prevent tooth decay. Fluoride is found naturally in foods that we consume, water we drink and most of the toothpaste we use. Within the body it is found mainly in bones and teeth. Normally the amount of this fluoride is small but high levels will lead to discolouring of teeth. It is therefore very important to know the level of fluoride in the body: if it is too high or too low. The amount in our body can be attributed to the level of fluoride in our food and other sources e.g. toothpaste, mouth rinses, or topical use from medications.

Would you like to take part? Please tick applicable box below

☐ Yes, I would like to take part

Comments _____

_____

_____

If you are happy to take part, please tick which kinds of the sample you are willing to provide

24-hour urine	☐
Spot urine	☐
Saliva	☐
Blood	☐
Nails	☐
Hair	☐

If you would like to take part or would be willing to be contacted to discuss whether you would be willing to be involved in this second part of the study, please complete the reply slip below and return it with your questionnaire to your child's school. A study pack will be sent to you containing more information about the research. If you are not interested in finding out more, please leave this session blank.

RESPONSE SLIP

Please tick as appropriate

I would like to receive more information before I decide whether or not I would like to take part in the study

Name: .....

Address: .....

.....

.....

Telephone: .....

Mobile: .....

Signature: .....

Please return the questionnaire and reply slips to your child's school

APPENDIX 18

STUDY INFORMATION DOCUMENT FOR ALFE PROJECT

What is this study information document (SID) for?

This SID contains detailed information about this project and the researcher involved. It also invites you to participate in the research project.

Who is doing the research?

This is part of a PhD research study conducted by Mr Idowu Oladipo Samson currently studying for a PhD degree in Teesside University, UK supervised by Dr Vida Zohoori from the School of Health and Social Care Teesside University, UK: and Dr Ruth Valentine and Dr Ralph Duckworth from the School of Dental Sciences, Newcastle University, UK.

What are we researching?

This research will find out the amount of fluoride that is retained in the body over long and short time periods among children of ages 4-5 and their parents aged 20-60 years using human biological tissues and fluids. By finding this out, we will provide information on which of the human tissues/fluids will be most reliable for estimation short term exposure and long term exposure to fluoride. In addition, we would find out how much fluoride is taken in by the children and their parents from food and drinks, how much of it is retained in the body and how much of it is excreted with the urine. The study will also find out the gene that is regulated by fluoride in the body.

Why are we researching fluoride biomarkers and what are the benefits?

Fluoride is mainly retained in the body by calcified tissues such as bones and teeth. It is found naturally in foods that we consume, the water we drink, and most toothpaste we use. Fluoride has been proven to be effective in the control of tooth decay (staining of teeth) in children at low concentration. However excessive exposure may lead to discoloration or mottling of teeth in some individuals. Monitoring of exposure to fluoride is therefore very important and biological markers like urine, hair, nails, saliva, and blood have proven to be very effective in the assessment of the amount of fluoride that is retained in the body over short and long periods of time capable of leading to the development of dental fluorosis (yellowing of teeth). This study will inform future research and health professionals on a standard biomarker that will useful for estimating to fluoride exposure and as a result we will be able to monitor it more efficiently.

What will the study include?

This study will include participants willing to provide samples of 24-hour urine, saliva, blood, hair and nails (fingernail and toenail) to estimate the amount of fluoride in the body. You would be given instruction on how to collect yourself the samples except for blood sample which will usually be collected at home by a qualified nurse recruited for the project. The researcher will visit you to collect the samples, in order to take them to the laboratory for analysis. DNA will also be extracted from the blood and taken to the UK for genetic analysis and the results will be kept in the biobank of Newcastle University, UK and no result will be fed back to participant.

Why have we asked you and your child?

You have been asked to take part in this study because you are the right age group for the study and living in either a high or low fluoride area of Plateau State, Nigeria.

Can you and your child participate?

The process of selecting you and your child will be based on the following criteria:

Inclusion criteria: you and your child can take part if you;

- are in the age categories 4-5 and 20-60 years old
- are able to consent to the study or to assent to the study for children aged 12-14years
- have no dietary restrictions and have good general and oral health
- are able and available to provide any of the samples listed above.
- have no chronic or metabolic disease and urinary infection
- have no oral disease (no tooth or gum pain) and no professional dental treatment such as the use of fluoride vanish for at least three months prior to the start of the study
- are willing to provide up to 4 teaspoons of blood for fluoride and gene analysis
- are willing for the extracted gene to be taken to the UK for analysis and stored in the biobank of Newcastle University, UK and the result will not be fed back to you.

Exclusion criteria: you and your child cannot take part if you:

- are using medication and have a restricted diet
- have metabolic disease or urinary infection
- have any oral disease
- are receiving professional dental treatment such as use of fluoride varnish for at least three (3) months prior to the start of the study
- have nail disease (for participant willing to provide nail sample) or are unwilling to remove nail varnish before sample collection.
- are using hair dye
- have a history of blood borne pathogens (e.g. viral hepatitis, HIV/AIDs) (for participants willing to provide blood samples).

What will happen if you decide to take part?

You will be asked to:

1. Complete a Food Frequency Questionnaire with the assistance of the researcher.
2. Collect you and your child's two full 24 hour urine samples
3. Collect your saliva
4. Collect your fingernail and toenail clippings
5. Collect a few strands of hair cut from the back of the head close to the scalp.

In addition, a qualified nurse will visit your home to collect about 4 teaspoons of blood (20 ml).

Will you need any special equipment?

All equipment required for the collection of any of the samples you are willing to provide will be given to you together with information on how to collect it. A laboratory technician will be available to collect the blood sample.

Urine collection sounds hard

It is not difficult by following our instructions. We will provide a jug for you to collect your urine and your child's in and a large screw top bottle to store it after collection. Thereafter, you should keep the bottle in a safe place prior to collection by the researcher on an agreed day.

What about saliva collection?

We will provide a graduated plastic tube and a funnel to ensure the saliva gets into the tube. You will place the funnel into the tube and expectorate into the funnel for 2-3 minutes.

What about nail collection?

We require you not to be using nail varnish in order for you to be able to provide clean nail samples. We will provide you with two zip bags to store fingernail and toenail clippings separately. You only need to cut all 10 nails (fingernails and toenails separately) and place the fingernails and toenails in separate zip bags.

What about hair collection?

You don't have to cut all your hair to provide a sample. You only need to cut a few strands from the back of the head close to the scalp. We will provide you with an envelope to store the hair, and the researcher will to pick it up on an agreed date.

Blood collection is painful!

Blood collection will not be as painful as you might think and it will be collected on the day agreed by a trained nurse in your home. The nurse will ask about your identity (ID number given to you), confirm the amount of blood that you agree to provide and collect it according to the standard medical procedure. The researcher will be there to pick up the blood sample. This will be used to analyse the fluoride content in the blood and also to allow DNA to be extracted. We will use the DNA to investigate genes that are regulated by F in the body. Separately from the study, you will be asked if we may store your DNA samples in an anonymized form, so that the sample cannot be identified as yours, for future studies that require the examination of genetic variability between individuals.

What will happen to the sample I provide?

All samples provided will be stored prior to analysis in the laboratory. Samples of urine, blood, saliva will be transported to the Federal College of Forestry, Jos Nigeria for the determination of fluoride. DNA (gene) will be extracted from the blood and the extracted gene, hair and nail samples will be transported to the UK for analysis. All samples provided will only be used for the analysis of the level of fluoride.

Do you need other information?

Yes, we will record your home address and age (in years). Teesside University, Middlesbrough, UK is sponsoring the study and appropriate insurance arrangements are in place.

Will the information obtained be kept confidential?

Yes, all details obtained from you and your child will be kept confidential. You will be given an ID number which will be written on all the documents provided to you. Likewise your child will be given a separate ID number if he/she is willing to take part. The ID will be used to keep all information anonymous, which will be held securely in strictest confidence at Teesside University, UK.

What is our involvement?

You will then be contacted by the researcher to arrange a visit to your home or child's school after an introductory session in your child's school: (1) to provide you with the information necessary for the study and collection equipment; (2) to obtain all your completed forms for the study and collect your samples:

Blood samples will be collected by a qualified laboratory technician at the hospital or your child's school. You will complete a questionnaire on how you feel about the way the samples are collected.

Do you have to take part?

No, participation in this study is completely voluntary. The researcher, Mr Idowu Oladipo from Teesside University, UK will be available to explain the purpose of the study to you and go through the information sheet as well. If you are willing to participate in the study, you will be asked to sign a consent form for yourself and for your child aged 4-5 years to take part except you feel your 4-5 year old child would be able to understand what is involved and decide for themselves, please then show them the information sheet for their age group and ask them if they would like to take part. If your child would like to take part, you would need to sign the consent form on their behalf. However, you and your child can withdraw at any stage during the 1st year of the study without giving a reason. All you need to do is call the researcher - Mr Idowu – whose contact details are found at the end of this document. Whenever you call, please quote your ID number or child's ID number found on the consent form and the information sheet.

If you have any question after taking part in the study?

Please feel free to contact the researcher Mr Idowu Oladipo if you are concerned about any aspect of the study on 08180458893 (Nigeria Research Mobile Number) who will be glad to answer any question you might have. You can also contact the Director of Studies Dr Vida Zohoori should you need further clarification on any aspect of the study on email v.zohoori@gmail.com at the School of Health and Social Care, University of Teesside, Middlesbrough, UK and Dr Alasdair MacSween PhD. BSc (Hon), MCSP, Principal Lecturer in Research Governance, Chair of School of Health and Social Care, Research Governance and Ethics Committee, Health and Social Care Institute, C1.10 Constantine Building, Teesside University, Middlesbrough, Tees Valley, TS1 3BA, UK. Tel +44 (0) 1642342965, fax +44 (0) 1642342983, email a.macsween@tees.ac.uk

Thank you for taking time to read this information sheet

APPENDIX 19

STUDY INFORMATION DOCUMENT

What is this study information document (SID) for?

This SID contains detailed information about this project and the researcher involved. It also invites you to participate in the research project.

Who is doing the research?

This is part of a PhD research study conducted by Mr Idowu Oladipo Samson currently studying for a PhD degree in Teesside University supervised by Prof Vida Zohoori from the School of Health and Social Care, Dr Ruth Valentine and Dr Ralph Duckworth from the School of Dental Sciences, Newcastle University.

What are we researching?

We are trying to evaluate the acceptability and reliability of collecting biological markers including urine, hair, nails, saliva and blood to assess exposure to fluoride among the following age groups: 4-5, 12-14, ≥20 years, in order to assess their strengths and weaknesses. By finding this out, we will provide information on which of the biomarker will be easily obtained without objection from donors for monitoring of exposure to fluoride. We would also like to know the biomarker that will be most effective for the estimation of exposure to fluoride among those listed.

Why are we researching fluoride biomarkers and what are the benefits?

Fluoride is mainly in the body with calcified tissues such as bone and teeth. It is found naturally in foods that we consume, water we drink and most of the toothpaste we use. Fluoride has been proven to be effective in the control of dental caries in children at low concentration. However excessive exposure will lead to discoloration or mottling of teeth. Monitoring of exposure to fluoride is therefore very important and biological markers have proven to be very effective in assessment of the amount of fluoride that is retained in the body. However, the acceptability of the collection procedure for these biomarkers is not known. This study will inform future research and health professionals on which of the biomarkers will be easily collectible without objection from potential donors and as a result we will be able to monitor fluoride exposure more efficiently.

What will the study include?

This study will include participants willing to provide any of 24-hour urine, saliva, blood, hair and nails (fingernail and toenail) to estimate the amount of fluoride in the body. You would be given instruction on how to collect yourself any of the samples you wish to provide except if you are willing to provide a blood sample which will usually be collected in any convenient place: home or work by a qualified nurse. The researcher will visit you to collect the samples you are willing to provide, in order to take them to the laboratory for analysis.

Why have we asked you and your child?

You have been asked to take part in this study because you are the right age group for the study.

Can you and your child participate?

The process of selecting you and your child will be based on the following criteria:

i. Inclusion criteria: you and your child can take part if;

- in the age categories 4-5, 12-14 and >20 years old

- able to consent to the study
- have no dietary restrictions and have good general and oral health
- able and available to provide any of the biomarkers listed above.
- have no gastrointestinal, bone, or renal problems or a history of urinary tract infection.
- Individuals willing to provide up to 5ml of blood (for participant willing to provide blood samples)

ii. Exclusion criteria: you and your child cannot take part if

- Receiving professional dental treatment such as use of fluoride varnish for at least three (3) months prior to the start of the study
- Has nail disease (for participant willing to provide nail sample)
- Unwilling to stop using hair treatment (dandruff shampoo, conditioner, hair spray, hair gel) about two (2) week before sample collection (for participant willing to provide hair samples).
- Individuals with a history of blood borne pathogens (e.g. viral hepatitis, HIV/AIDs) due to laboratory restriction (for participant willing to provide blood samples).

What will happen if you decide to take part?

You will be asked to:

1. Complete a fluoride exposure questionnaire and a medical history
2. Provide any of the samples you are willing to give, which may include collection of:
    a. two 24 hour urine samples
    b. saliva
    c. fingernail and toenail clippings
    d. a few strands of hair cut from the back of the head close to the scalp.

In addition, if you are willing to provide blood, a nurse will visit your home or work to collect it. Where sample have been arranged to be collected in the office, participants must obtain their employers permission before arranging for the sample collection.

Will you need any special equipment?

All equipment required for the collection of any of the samples you are willing to provide will be given to you together with information on how to collect it. A nurse will be available to collect the blood sample.

Urine collection sounds hard!

It is not difficult by following our instructions. We will provide a jug for you to collect your urine and your child's in and a large screw top bottle to store it after collection. Thereafter, you should keep the bottle in a safe place prior to collection by the researcher on an agreed day.

What about saliva collection?

We will provide a graduated plastic tube and a funnel to ensure the saliva gets into the funnel. You will place the funnel into the tube and expectorate into the funnel for 2-3 minutes.

What about nail collection?

We require you not to be using nail varnish in order for you to be able to provide nail samples. We will provide you with two zip bags to store fingernail and toenail clippings separately. You only need to cut all 10 nails (fingernails and toenails separately) and place the fingernails and toenails in separate zip bags.

What about hair collection?

You will stop using shampoo for about two (2) weeks prior to sample collection. You don't have to cut all your hair to provide a sample. You only need to cut a few strands from the back of the head close to the scalp. We will provide you with an envelope to store the hair, and the researcher will to pick it up on an agreed date.

Blood collection is painful!

Blood collection will not be as painful as you might think because it will be collected on the day agreed by a trained nurse. The nurse will ask about your identity (ID number given to you for the research), confirm the amount of blood that you agree to provide and explain the procedure. Verbal consent will be obtained before sample is collected. Then the nurse will expose the site of collection in the arm and apply a tourniquet to the upper arm and identify a vein in the ante-cubital fossa where the needle will be inserted through the skin and ten into the vein. The needle will be held firmly in the vein with one hand and appropriate blood tube will be inserted into the barrel to collect the blood sample. After obtaining the blood, the nurse will remove the specimen tube gentle whilst holding the needle firmly in place. The nurse will then release the tourniquet and place a dry cotton wool ball over the needle when withdrawing the needle. The specimen will be completely and accurately labelled (date, ID code) and stored in the appropriate bag which will be kept in the waterproof box used in transporting to Teesside University where analysis of fluoride will be conducted. The nurse will ensure the wound has stopped bleeding and apply a plaster on the puncture site. **The researcher will be there to pick up the blood sample to be analysed for fluoride in Teesside University laboratory.**

Do you need other information?

Yes, we will record the first three digit (e.g. TS1) of your post code and age (in years).

Will the information obtained be kept confidential?

Yes, all details obtained from you and your child will be kept confidential. You will be given an ID number which will be written on all the documents provided to you. Likewise your child will be given a separate ID number if he/she is willing to take part. The ID will be used to keep all information anonymous, which will be held securely in strictest confidence at Teesside University.

What is your involvement?

You will then be contacted by the researcher to arrange two visits to a convenient place (home, work etc.): (1) to provide you with the information necessary for the study and any collection equipment depending on which biological marker you are willing to provide. (2) to obtain all your completed forms for the study and collect your samples. If you choose to be visited at work premises for sample collection, you will have to obtain your employers permission before arranging sample collection.

Visit one: Before the study starts, we will visit to answer any questions you have about the study and as well ask some questions about you and your child's general health. During this visit we will give you: 1) an informed consent form and assent form if your child is willing to participate; 2) a fluoride exposure questionnaire (including information about diet, previous dental history, use of supplements); 3) depending on the biomarker you are willing to provide, we will give you the corresponding collection kit.

Visit two: We will visit you again to collect your samples and completed fluoride exposure questionnaire. If you are willing to provide a blood sample, a nurse will be available to collect it.

Do you have to take part?

No, participation in this study is completely voluntary. The researcher, Mr Idowu Oladipo from Teesside University will be available to explain the purpose of the study to you and go through the information sheet as well. If you are willing to participate in the study, you will be asked to sign a consent form. If your child is 12-14 years old and you are happy for your child to be asked if they want to take part and to take part if they choose to, you will pass on the Study Information specifically for 12-14 year old and assent form to your child. If your child is willing to participate in the study after careful consideration of the document, he or she will be asked to sign the assent form. However, you and your child can withdraw at any stage of the study without giving a reason. All you need to do is call the researcher - Mr Idowu – whose contact details are found at the end of this document. Whenever you call, please quote your ID number or child's ID number found on the consent form and the information sheet.

If you have any question after taking part in the study?

Please feel free to contact Idowu Oladipo if you are concerned about any aspect of the study on 01624384157 or o.idowu@tees.ac.uk who will be glad to answer any question you might have. You can also contact Prof Vida Zohoori should you need further clarification on any aspect of the study on 01642342937 or v.zohoori@tees.ac.uk at the School of Health and Social Care, University of Teesside, Middlesbrough, UK.

Thank you for taking time to read this information sheet

APPENDIX 20

SAMPLE COLLECTION INSTRUCTION

Why do we collect human biological samples?

You and your child consume food and drink that contain fluoride and this fluoride is absorbed in the body

The fluoride will leave the body through urine and some of it will be retained in the body among various tissues e.g. bone, blood, saliva, hair, nails etc.

Measuring the amount of fluoride will help us to know how much fluoride has been absorbed during consumption and retained within the body. This can be achieved by analyzing blood, saliva and 24-hour urine, hair and nails.

24-hour urine *collection general instruction*

All urine that is passed in a 24-hour period should be collected as it is important for our analysis. Only collect urine in the container provided by the researcher. These containers have been evaluated and found to be clear of contamination. Do not use any other container.

- You are provided with a bottle, a funnel and a jug to collect the urine samples.
- In the morning, at the start of 24 hour collection, you will discard your first urine of the day (**DO NOT COLLECT THIS URINE INTO THE STORAGE CONTAINER**). You should record this date and time in the urine record form.
- For the next 24 hours, **YOU will** collect all urine passed each time you urinate up to the time you go to bed on the same day and including the first time you urinate on the next day at the same time you started the 24-hour collection the previous day.
- Collect the urine in the disposable jug and then transfer it into the storage bottle with the funnel that you will be given. Repeat this process each time you urinate.
- Store the bottles of urine in a convenient place out of reach of children and be sure to replace the safety cap on the bottle after each sample is added.
- You would record the time and date of the last urine.
- You would be asked if it is a complete sample and in the case of incomplete urine collection, you would be asked if you are willing to repeat the procedure.

Spot urine collection

- You are provided with disposable cups, transfer funnel and storage tubes.
- You would urinate into the disposable cups at set times during the day and you would then transfer a small amount into the storage tube using the transfer funnel.
- Store the tube until the researcher visits to pick it up.

Saliva collection

- You are provided with a collection plastic tube with a lid.
- At the time of collection, you will first swallow the saliva in your mouth and then, as your mouth starts to fill up again, you allow the saliva to flow into the tube for 2-3 minutes.
- Store in a secured place until the researcher's visit.

Hair collection

- You are provided with a zip bag for storage. On the day of collection, wash and dry your hair.
- You will gather a few strands of hair) from the back of the head and cut hair near the scalp (close to the head) with metal scissors or any cutting equipment you are comfortable with, cutting at least 5 cm (the length of the thumb or shorter).
- Do not cut the scalp or pull hair from the scalp and if hair is short, you should cut enough hair equal to 5cm in length as described above.
- Place hair in the storage bag.

Note: This should be done with the assistance of a partner/parent/guardian

Nail collection

- You are provided with two zip bags for storage
- Before collection, you will wash and dry your hands and feet with soap.
- You will then cut all 10 nails, fingernail and toenails separately.
- Place the fingernail and toenail clippings in separate zip bags.

Note: it is important that you separate fingernails from the toenails and nails must be free of polish

Blood collection

This will be done by a qualified laboratory technician and the sample will be collected according to standard medical practice just like that obtained in the hospital.

When will we pick up the samples from you?

The samples will be picked up from you at a place and time agreeable to you. The researcher will visit your home to pick up the samples at an arranged date or you would be told to bring the sample to the hospital.

What will be done to the samples provided?

The urine samples will be taken to the hospital laboratory where the total volume will be measured, a small quantity will be immediately analyzed for fluoride concentration and any remaining will be discarded according to the standard procedure. The hair and nail samples will be transported to a laboratory in the University of Teesside, UK where they will be analyzed for fluoride. A blood collected will be divided into two portions. Plasma will be separated from the first portion and analyzed for fluoride at the hospital laboratory and to the second, DNA will be extracted will be extracted from the second portion blood sample and the blood sample is disposed according to standard laboratory procedure. The extracted DNA then will be transported to the UK for identification and banked for future use.

Do you have any problem?

Please don't hesitate to contact Mr. Idowu on the research mobile number 08180458893. If you have any problem collecting any of the samples, he will be happy to help.

APPENDIX 21

Table 1: Field work in Nigeria (High fluoride area)

S/N	Task	April				May				June				July			
		Week 1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	Arrival into Nigeria																
1	Sourcing chemicals/ Purchase of lab consumables																
2	Visit to study site I																
3	Visit to schools for invitation to participate																
4	Recruitment of participant 20 participants/week (10 adults and 10 children)																
5	Analysis of sample/ Blood DNA extraction (water, saliva, urine and plasma)																

Table 2: Field work in Nigeria (Low fluoride area)

S/N	Task	May				June				July				August		
		Week 1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
1	Visit to schools for invitation to participate															
2	Recruitment of participant 20 participants/week (10 adults and 10 children)															
3	Analysis of sample/ Blood DNA extraction (water, saliva, urine and plasma)															
4	Food analysis															
5	Travel to the UK															

Table 3: Weekly activities (Sample collection, preparation and analysis) for 20 participants: 10 adults and 10 children

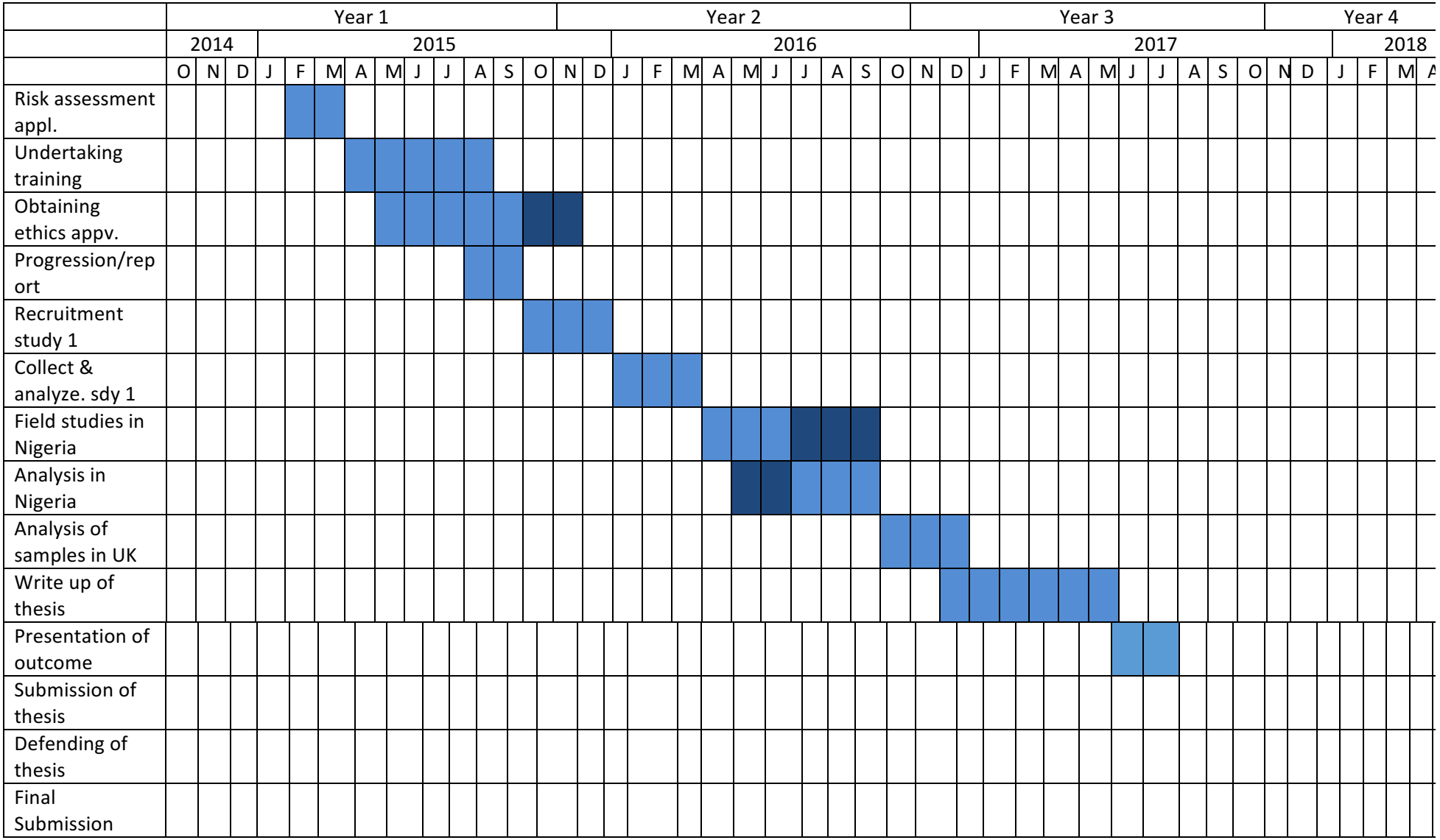
S/N	Task	Mon		Tues		Wed		Thurs		Fri		Sat		Sun	
		AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
1	Visit 1: Introductory session in school: Assess suitability (Medical questionnaire), obtain consent, measure height and weight														
2.	Participant to collect 24 hrs. urine, nail, hair and food samples														
3	Visit 2: In school to collect blood (Parent/child) and FFQ assessment and retrieval														
4	Transportation to the laboratory/separate plasma and DNA extraction from blood														
5	Analysis of plasma, expectorated saliva/saliva and urine samples			PS pre.	DU & SA analysis	PS analysis	Recovery (all samples)	WA/drinks analysis	Recovery (plasma) analysis						
6	Visit to homes to collect DU/SA														

Table 4: Sample analysis in the UK

S/N	Task	July				August				September				October			
		Week 1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	Arrival to UK																
2	Preparation of chemical and reagent																
3	Analysis of nail samples (n=120) (60 samples/week)																
4	Analysis of hair samples (120) (60 samples/week)																
5	Analysis of tooth paste (n=?)																
6	Reanalysis of samples																
7	Calculation of data and analysis of result																

Table 5: Research study time plan

	Year 1															Year 2												Year 3												Year 4			
	2014			2015												2016												2017												2018			
	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A
Induction/ prop.																																											
Literature search																																											
Develop study protocol																																											
Preliminary survey																																											


Year 1
Year 2
Year 3
Year 4
2014
2015
2016
2017
2018
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A
Risk assessment appl.
Undertaking training
Obtaining ethics appv.
Progression/report
Recruitment study 1
Collect & analyze. sdy 1
Field studies in Nigeria
Analysis in Nigeria
Analysis of samples in UK
Write up of thesis
Presentation of outcome
Submission of thesis
Defending of thesis
Final Submission

APPENDIX 22

Low fluoride area

Food/Drink	Fluoride concentration (mg/l, mg/kg)	Source
Egg boiled	0.57	UK database
Herbal teabag	12.00	UK database
Coffee	7.00	UK database
Fruit juice	10.20	UK database
Cheese	8.25	UK database
Soya milk	30.70	UK database
Cow milk	0.80	UK database
Paster	57.00	UK database
Noodles	10.70	UK database
Cornflakes	7.95	UK database
Plantain	0.77	UK database
Beef roasted	6.00	UK database
Beef grilled	5.60	UK database
Beef pie	7.50	UK database
Mango juice	3.30	UK database
Pear raw	1.80	UK database
Pineapple canned	0.04	USDA database
Melon	0.60	UK database
Oranges	2.30	UK database
Cashew	0.13	Farias *et al*. 2014
Guava	0.08	Farias *et al*. 2014
Tap water	0.70	Akpata *et al*. 2009
River/Stream water	0.14	Akpata *et al*. 2009
Bottled water	0.30	Ajaji *et al*. 2000
Sachet water	0.17	Ajaji *et al*. 2000
Tubber	0.289	Ibiyemi 2016

UK database, Zohoori and Maguire, 2014(µg/100g).

US database, Cutrufelli et al., 2004.

High fluoride area

Food/Drink	Fluoride concentration (mg/kg, mg/l)	Source
Egg boiled	0.57	UK database
Herbal teabag	85.30	UK database
Black tea	193.30	UK database
Coffee	83.00	UK database
Chocolate	23.00	UK database
Fruit juice	10.20	UK database
Cheese	8.25	UK database
Soya milk	30.70	UK database
Cow milk	0.80	UK database
Rice	175.70	UK database
Wheat	7.2	Leading Edge Research group
Paster	293.00	UK database
Noodles	41.30	UK database
Cornflakes	7.95	UK database
Potatoes (boiled)	85.00	UK database
Plantain	0.77	UK database
Beef roasted	6.00	UK database
Beef grilled	5.60	UK database
Beef pie	7.50	UK database
Mango juice	3.30	UK database
Pear raw	1.80	UK database
Pineapple canned	0.04	UK database
Melon	0.60	UK database
Oranges	2.30	UK database
Cashew	0.13	Farias *et al*. 2014
Guava	0.08	Farias *et al*. 2014
Tap water	0.70	Akpata *et al*. 2009
River/Stream water	0.14	Akpata *et al*. 2009
Bottled water	0.30	Ajaji *et al*. 2000
Sachet water	0.17	Ajaji *et al*. 2000

Food/Drink	Fluoride concentration (mg/kg, mg/l)	Source
Tubber	0.289	Ibiyemi 2016

UK database, Zohoori and Maguire, 2014(µg/100g).

US database, Cutrufelli et al., 2004.

APPENDIX 23

EFFECT ON GENETICS ON FLUORIDE METABOLISM

Evidence has revealed contribution of certain genetic component in the pathogenesis of dental fluorosis following the prevalence of dental fluorosis during amelogenesis among children receiving optimum amount of fluoride despite the decline in dental caries (Williams and Zwener, 1990), participants with comparable level of fluoride intake (Yoder *et al*., 1998) and among ethnic groups with different degree of susceptibility (Butler *et al*., 1985). Everett *et al*. (2002) revealed differences in susceptibility and resistance among 12 inbred strain of mice with rapid onset and development of dental fluorosis in the A/J mice strain while the 129P3/J mice strain is least affected with minimal dental fluorosis. This was confirmed by Carvalho *et al*. (2009) who reported that the teeth of the 129P3/J mice are more resistant to the effect of fluoride compared to the A/J strain despite the fact that both strains were different with respect to fluoride metabolism and amelogenesis. However, the resistant strain (A/J strain) show an increase in urinary fluoride excretion and subsequently lower plasma concentration as well as lower femur fluoride level contrary to what was expected. A further study on bone properties in inbred mouse strains including A/J, SWR/J and 129P3/J exposed to increasing fluoride doses showed significant alterations in bone quality of the A/J strain and moderate alteration in the SWR/J strain whereas no effect was found on the 129P3/J strain (Mousny *et al*., 2006; Claudia *et al*., 2013). The first human evidence was reported by Huang *et al*. (2008) who investigated PvuII and RsaI polymorphisms in the COL1A2 gene among 8 and 12 years old with and without fluorosis in China and showed a significantly increased risk of dental fluorosis in children with the homozygous genotype PP of COL1A2 PvuII compared to children with the homozygous genotype pp in an endemic fluorosis village but no association of polymorphism of genes on bone health and sensitivity to fluoride (Ba *et al*., 2009). Genes involved in enamel and dentin formation, diet preference, tooth morphology, salivary flow, and composition might have an influence on caries risk (Viera *et al*., 2008; Wang *et al*., 2012). More still need to be known on the physiological, biochemical and molecular mechanism lurking with the resistance of the genes particularly with COL1A2 gene, since other gene tested (ER Rsal genotype) did not show correlation with dental fluorosis when investigated among 8-12 years old children with or without dental fluorosis in China (Wang *et al*., 2009). The aim of the study was to investigate the prevalence of a single nucleotide polymorphism within the COLIA2 gene among people living in low and high fluoride areas.

Whole blood sample preparation and analysis

The researcher separates 2ml of each whole blood sample from blood sample of participants who consented to the DNA collection from the fluoride biomarker studies. The sample was transferred to

another tube and stored at -20^{o}C at the Plateau State Institute of Virology, Jos Nigeria prior to DNA analysis.

- **Purification of genomic DNA from whole blood**

The frozen samples were thawed leaving them at room temperature. The buffers, including Lysis Buffer, Wash Buffer, and Proteinase K Buffer PR, were prepared according to the manufacturer's direction prior to the commencement of the extraction. The incubator temperature was adjusted to 70^{o}C and Elution Buffer was preheated to 70^{o}C. Lymphocytes from whole blood were separated by lysing the red blood cells (RBCs) using a hypotonic buffer (Proteinase K solution). Twenty-five (25µl) of Proteinase K solution was added to 200 µl blood sample in a 1.5 ml microcentrifuge tube with 200 µl Lysis Buffer and mixed by vortexing vigorously for 10-20 s and incubating at 70^{o}C for 10-15 min with a thermomixer. The DNA binding conditions were adjusted by adding 210 µl ethanol (96-100%) and mixed by vortexing for 5 min. Each sample was then loaded onto the ISOLATE II Blood DNA spin column placed in a collection tube and centrifuged at 11,000 g for 1 min in order for the DNA to bind. The process was repeated until all the sample was completely filtered through the matrix, after which the column was transferred into a new 2 ml collection tube. To the column, 500 µl Wash Buffer (GW1) was added and centrifuged at 11,000 g for 1 min, after which the column was transferred into a new 2 ml collection tube where 600 µl of Wash Buffer (GW2) was added and then centrifuged at 11,000 g for 1 min. The flow through was then discarded from the collection tube and the column was replaced. Spin Column with silica memrane was then centrifuged at 11,000 g for 1 min to remove residual ethanol. The ISOLATE II Blood DNA Spin Column was then placed in a 1.5 ml micro centrifuge tube and 100 µl preheated Elution Buffer G (70^{o}C) was added directly onto the silica membrane of the Spin Column, then allowed to incubate at room temperature for 1 min and centrifuged at 11,000 g for 1 min.

- **Quantification of the extracted DNA with UV spectrophotometry and purity determination**

A quantitative spectrophotometric assay of DNA was performed using a Nano-drop UV spectrophotometer (ND-1000, Germany). The purified genomic DNA was defrosted and thoroughly mixed, then 1 µl sample of the extracted DNA was placed in the spectrophotometer well. Absorbance was measured at wavelengths of 260 and 280 nm (A_{260} and A_{280}), respectively. The absorbance ratio provides an estimate of DNA purity. An absorbance ratio of >1.8 and <2.0 was considered to indicate a good, purified DNA. Also, a ratio of <1.8 is indicative of protein contamination, while a ratio of >2.0 indicates RNA contamination.

- **Identification of genomic DNA by a PCR Assay: PCR Amplification**

The adequacy of blood DNA extracts for the PCR-based assay was assessed by amplifying the gene of inherent COL1A2 and a reference gene (GAPDH). PCR was carried out in 25 µl total reaction volumes, each containing 1 µl primer (for each sample: 0.5 µl primer for the forward reaction and 0.5 µl for the reverse reaction), 5 µl PCR master mix used for PCR analysis, 10 µl DNA sample and made up with 9 µl nuclease-free water, and centrifuged. The reaction mixture was heated to 95^{o}C for 5 min, followed by 29 cycles, each consisting of 30 s denaturation at 95^{o}C, 30 s annealing at 58^{o}C, 1 min extension at 72^{o}C and a final 5 min extension at 72^{o}C. The PCR amplification products (10 µl) were subjected to electrophoresis on a 1.5% agarose gel in 1 X Tris-acetate-EDTA buffer (75 g of agarose and 50 ml of 1xTAE Buffer) at 80 V for 30 min and stained with gel red (SYBR Safe). Gels were visualized on a translator under UV light and images obtained from the presence of COL1A2 gene.

Results

To evaluate the purity, the absorbance was measured to detect other possible contaminants. A good quality DNA will have an A_{260}/A_{280} ratio of 1.8-2.0. A lower or higher ratio indicates more contaminants are present. DNA absorbs light most strongly at 260 nm (A_{260}) but DNA is not the only molecule that absorbs UV light at 260 nm, RNA and guanidine also absorb there. In addition, aromatic amino acids absorb at 280 nm. In this low fluoride area, 28.6% of the DNA samples from both adults and children are good quality with absorbance ratios in the optimum range whereas 57.1% (adults and children) are of poor quality with absorbance ratios < 1.8. The concentration of the DNA samples range from 2.0 to 62.4 ng/µl in adults while in children the range is between 3.2 to 133.7 ng/µl. Images of COL1A2 from the PCR were not very visible for identification.

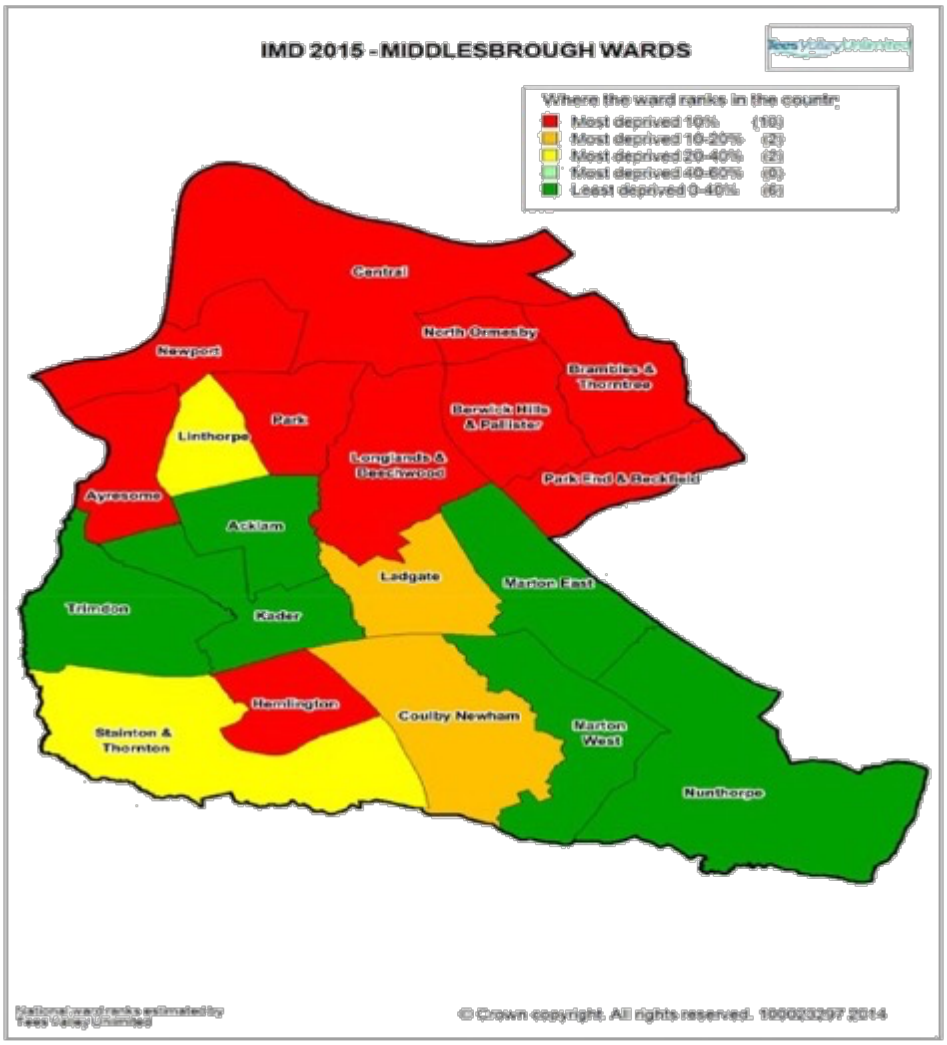
Figure 1. A representative gel image of PCR products run on a 1% agarose gel.

Figure 1 show the image taken when DNA was vitualised on a UV trans-illuminator. The band show a positive reaction to the reference gene (GAPDH).

Discussion

Due to the poor quality of the extracted DNA samples, the researcher did not progress to PCR amplification and gene sequencing. The poor quality could be associated to the difficulty in storage encountered during transportation of samples from Nigeria to the UK.

APPENDIX 24



Courtesy

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/465791/English_Indices_of_Deprivation_2015_-_Statistical_Release.pdf

APPENDIX 25

GOVERNMENT OF PLATEAU STATE

MINISTRY OF HEALTH HEADQUARTERS

P.M.B. 2014, JOS, PLATEAU STATE

PUH/209/VOL.II/X

4th March, 2016

Mr. Idowu Oladipo
Teesside University,
School of Health and Social Care

RE: APPLICATION FOR APPROVAL OF RESEARCH STUDY.

Reference to the above subject matter.

I have been directed to convey clearance for you to conduct research on the topic "*Assessment of contemporary and recent biological makers of human exposure to fluoride in Nigeria*".

You are to submit a copy of the research outcome to Plateau State Ministry of Health, since the study will cover Bokkos, Barkin Ladi and Langtang North LGAs.

Thank you.

Paul D. Dwagas
Director Planning
For: Hon. Commissioner

APPENDIX 26

Evidence of dental caries among children in the low fluoride area (0.04 mg F/l)

Evidence of dental fluorosis among children in the high fluoride area

Evidence of dental fluorosis among adults in the high fluoride water area

APPENDIX 27

CONTROL ID: 2951331
TITLE: Fingernails and Toenails Good Biomarkers of Chronic/Sub-chronic Fluoride Exposure.
PREFERRED PRESENTATION TYPE: Oral
CURRENT SCIENTIFIC GROUPS & NETWORKS: Pharmacology/Therapeutics/Toxicology
PRESENTER: Oladipo Idowu
PRESENTER (INSTITUTION ONLY): Teesside University
AUTHORS/INSTITUTIONS: O.S. Idowu, V.F. Zohoori, R.M. Duckworth, School of Health and Social Care, Teesside University, Middlesbrough, Cleveland, UNITED KINGDOM|R.A. Valentine, School of Dental Sciences, Newcastle University, Newcastle, UNITED KINGDOM|
ABSTRACT BODY:
Objectives: This study aimed to investigate the relationship between fluoride concentration in fingernails (F_{FN}) and toenails (F_{TN}) (µg/g) and i) total daily fluoride intake (TDFI), ii) drinking water fluoride concentration (F_W).
Methods: Sixty healthy children (4-5y) and their parents (≥20y) in low- and high fluoride water areas (LFA, HFA, respectively), in Nigeria, participated. Total daily dietary fluoride intake (TDDFI) was assessed by food frequency questionnaire coupled with analysis of fluoride content of food and drinks consumed. Toothpaste ingestion was assessed through questionnaires. TDFI was calculated from TDDFI and toothpaste ingestion. Fingernail and toenail clippings were cleaned in a sonicator with double-distilled ionized water and oven dried. Fluoride concentrations of diet and nail clippings were measured by fluoride-ISE after overnight HMDS-facilitated diffusion.
Results: TDFI [mean(SD)] for children and adults respectively was 0.075(0.036) and 0.036(0.020) mg/kgbw/d in LFA (F_W=0.04 mg/L) and 0.277(0.184) and 0.125(0.093) mg/kgbw/d in HFA (F_W=3.05 mg/L). Mean(SD) F_{FN} was 3.23(2.63) and 2.80(1.77) µg/g in children and adults respectively in LFA and 12.58(7.05) and 9.41(3.74) µg/g respectively in HFA, whereas mean(SD) F_{TN} was 4.07(3.72) and 3.28(2.82) µg/g in children and adults respectively in LFA and 11.38(5.23) and 10.20(5.79) µg/g respectively in HFA. Moderate, positive and statistically significant correlations were found between F_{FN} and TDFI in children (Pearson's correlation (PC) = 0.448, p=0.002) and in adults (PC=0.506, p<0.001). Positive and statistically significant correlations were found between F_{TN} and TDFI in children (PC=0.488, p<.001) and in adults (PC=0.502, p<0.001). F_{FN} and F_{TN} showed strong positive and statistically significant correlations with F_W for both children and adults.
Conclusions: Fingernail and toenail clippings may be used as a biomarker of chronic fluoride exposure due to the good relationship established with TDFI.
TABLE TITLE: (No Tables)
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TABLE FOOTER: (No Tables)
(No Image Selected)
KEYWORDS: Biomarker, Exposure, Fluoride, Fingernail, Toenail.
AWARDS: IADR-BSODR Division-Senior Colgate Prize|IADR-BSODR Division-Unilever Poster Prize
Student Status - Abstracts: PhD Student (no professional degree)
Support Funding Agency/Grant Number - Abstracts: (none)
Financial Interest Disclosure: NONE
Group Author Abstracts - Abstract: (none)

clared, in 8 brands a fluoride concentration ranging from 200 to 225 mg F/kg was declared and in one fluoridated brand the concentration was not declared. Six aliquots of each package were weighed (2.5 g ± 0.01) and dissolved in the proportion of 0.025 g/mL of purified water. Duplicates of 1.0 mL of the salt solutions prepared were buffered with the same volume of TISAB II. Fluoride concentration in the mixture was determined with ion specific fluoride electrode calibrated with standards solutions ranging from 0.25 to 16.0 µg F/mL, which were mixed with TISAB II (1:1; v/v) containing 0.025 g NaCL/mL. Results were expressed in mg F/kg salt (w/w). In the two non-fluoridated brands only traces of fluoride were found. In the nine fluoridated brands, two presented only traces of flaoride (mean ± SD; n=3) (1.8 ± 0.1; 18.6 ± 13.1.), five brands showed fluoride concentration below the local regulation (131± 34.3; 180.6±12.3; 184.6 ± 34.8; 190 ± 47.2; 199 ± 18.9) and two brands contained a fluoride concentration according to the Nicaraguan law (209.8 ± 48; 211.4 ± 26). Considering the inconsistencies found in fluoride concentration and in labeling, the findings suggest that the surveillance system of salt fluoridation program in Nicaragua should be improved.

tinal tubules, which could lead to an alleviation of hypersensitivity in vivo.

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Hair: A Biomarker of Chronic/Sub-chronic Fluoride Exposure

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Despite the suggested use of hair as a non-invasive biomarker of chronic/sub-chronic fluoride (F) exposure, it has not been fully investigated. This study aimed to investigate the correlations between F concentration in hair (FHair) and i) drinking water F (FWater) and ii) urinary F excretion (UFE) in children and adults. Healthy children aged 3–5y and their parents aged >20y were recruited in low-fluoride (LF) and high-fluoride (HF) areas of Nigeria. UFE was measured in voided urine collected over a 24-hr period and FHair was measured in hair swatches. Fluoride concentration of urine and water samples was measured directly using a F-ion-selective electrode after adding TISAB and of hair samples using a standard HMDS-diffusion method. In total, 56 children and 56 adults provided valid samples: 28 children and 28 adults receiving LF water, mean (SD) = 0.04 (0.20) mg F/l, and the same number receiving HF water, 3.05 (1.11) mg F/l. Mean (SD) UFE for adults and children, respectively, was 0.140 (0.081) and 0.300 (0.143) mg/kg body weight/d in the HF area and 0.005 (0.003) and 0.004 (0.002) mg/kg body weight/d, respectively, in the LF area. Mean (SD) FHair was 5.691 (3.182) and 1.831 (1.091) µg/g for adults and children, respectively, in the HF area and 1.368 (1.062) and 0.743 (0.609) µg/g, respectively, in the LF area. A moderate statistically significant correlation (Pearson Correlation = 0.510, $p<0.001$) was found between FHair and FWater; whereas the corresponding correlation between FHair and UFE was weak (Pearson Correlation = 0.275, $p<0.005$). The statistically significant positive correlation between FHair and FWater indicates that hair can be used as a non-invasive biomarker of chronic/sub-chronic fluoride under conditions where local customs permit.

(ArgFD:243.0±23.7a and FD:363.8±32.8b) ($p=0.002$), but no difference on %SHL was found between ArgFD and FD (ArgFD:37.4±3.3a and FD:29.2±4.5a) both irrespective to sucrose concentration. Although reducing IEPS on dental plaque, ArgFD use presented similar anti-caries effect compared to regular FD use when plaque was exposed to sucrose 4x or 8x/day during 14 days.

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Mineral Ions Released from Experimental Toothpaste Containing S-PRG Filler Enhance Fluoride Retention in Oral Biofilm

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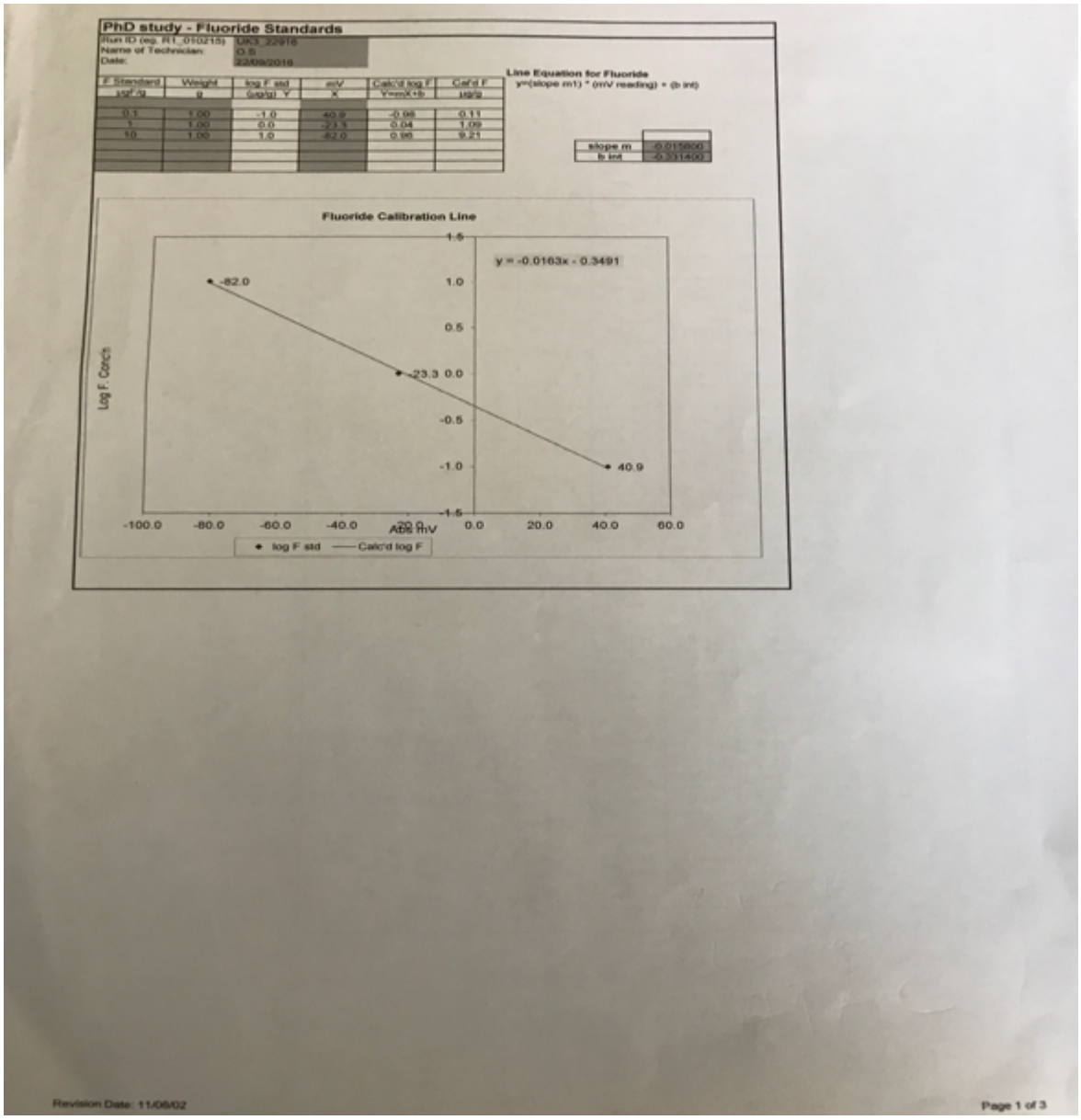
APPENDIX 28



PhD study - Fluoride Standards

Run ID (eg. R1_010215): DR3_22916
Name of Technician: D.S
Date: 22/09/2016

Line Equation for Fluoride
y=(slope m1) * (mV reading) + (b int)

F Standard ug/ml Y	Weight g	log F std (ug/ml) Y	mV X	Calc'd log F Y=mX+b	Calc'd F ug/g
0.1	1.00	-1.0	40.9	-0.96	0.11
1	1.00	0.0	-23.3	0.04	1.09
10	1.00	1.0	-82.0	0.96	9.21

slope m	-0.015800
b int	-0.331400